

**AIR POLLUTION EPIDEMIOLOGY:
CONSIDERATIONS IN TIME SERIES MODELING**

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Abbreviated Title: Time Series Modeling

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ABSTRACT

Recent epidemiological studies have indicated that ambient air pollution, including PM₁₀, is associated with excess mortality and morbidity. These studies have included both cross-sectional comparisons across communities and time-series analyses over time in single communities. Time-series analysis offers certain advantages, primarily in that the study population is the same over time, so that it acts as its own "control". However, modeling such data is complicated by the fact that other environmental factors and other causes of illness can confound the results unless they are adequately addressed. For example, wintertime influenza epidemics cause long-wave peaks in respiratory mortality, and variations in emissions, dispersion, and atmospheric chemistry can cause seasonal cycles in pollution. Such superimposed long-wave variations in both health outcomes and pollutant concentrations can undermine the statistical validity of time-series models by inducing autocorrelation, and can create long-wave "noise" signals which can overwhelm a short-term "signal" of interest. Also, model specification can strongly affect the results of a time-series model. For example, analyses focusing on only one routinely collected pollution metric, to the exclusion of other possibly more influential pollution components, can cause the effects of the overlooked pollutants to be ascribed to the studied pollutant. In addition, the potential effects of non-normal (e.g. Poisson) data distributions on time-series results need to be considered. It is concluded that how these various time-series modeling factors are, or are not, addressed can have a large influence on the study conclusions, or the "message" resulting from such analyses. Sensitivity analyses incorporating multiple modeling methods and model specifications are therefore recommended as part of such an analysis. Moreover, exploratory and diagnostic procedures are recommended in this paper which may aid the modeler in assessing and avoiding the noted problems, and which will allow the validity of such studies to be more easily documented and inter compared.

INTRODUCTION

Extreme historical pollution episodes have had obvious health consequences, so quantifying the human health effects in those cases was straightforward. For example, the London Fog pollution episode in December 1952 was associated with approximately 4000 excess deaths over a period of just weeks (Min. of Hlth. of Gr. Britain, 1954). At more routine pollution levels, however, other factors (e.g. seasonal changes and viral epidemics) often dominate morbidity and mortality variations over time, and the effects of pollution are harder to discern. Only via careful statistical modeling can pollutant effects be quantified at more "normal" pollution levels.

Time-series analysis has become a widely applied statistical method to evaluate the health implications of ambient air pollution exposures, as it offers certain advantages over more traditional cross-sectional studies. The primary advantage of time-series analysis is that health and pollution variations of the same population is studied over time, so that it acts as its own "control" population. This obviates the need to separately analyze comparison populations, and all their respective associated (and potentially confounding) differences in characteristics.

Time-series analyses have their own challenges, foremost of which is that they are often complicated by the fact that other environmental factors, such as seasonal variations, may confound the model results. Indeed, the seasonality of morbidity and mortality was explicitly mentioned as an important medical factor to be accounted for as long ago as 400 B.C. by Hippocrates in his treatise "On Airs, Waters, and Places". This potential complication to the evaluation of pollution health effects can, however, be addressed by various available statistical techniques, each with inherent advantages and disadvantages. As shall be shown, alternative time-series modeling approaches and model specifications can yield dramatically differing conclusions, especially if the model applied is inappropriate to the particular situation. If it is possible for a statistical model to determine the nature of the results derived, then, in such cases, "The model is the message" in the statistical interpretation of the epidemiological data, analogous to the way Marshall McLuhan argued in the Sixties that "The medium is the message" when people process information. This paper will attempt to outline some of the modeling aspects which are most critical to consider in evaluating time-series studies, and discuss their respective implications to the interpretation of such analyses.

TIME-SERIES MODELING CONSIDERATIONS

In order to have meaningful time-series results, it is important to make sure that the model is not adversely influenced by factors which may confound or statistically invalidate its results. These factors may include: long wave cycle influences on the data; model specification choice; and data distribution effects. These model factors are considered individually below, with illustrative examples.

Long Wave Cycles

Long wave cycles are important to consider because seasonal cycles and other variations in the health outcome data having periodicities longer than a few days in length (e.g. viral influenza epidemics and day-of-week patterns) result in long wave autocorrelation. Autocorrelation in a model can obscure the pollution-health association to be evaluated by creating long-wave "noise" signals which can overwhelm a short-term "signal" of interest. For example, respiratory mortality rates in the U.S. are generally higher in winter than in summer. Likewise, pollutants usually also show seasonal variations in concentration and, depending on whether or not they coincide or run contrary to the mortality cycle, this can bias the pollutant relationship upward or downward, respectively. Even within a single season, these annual cycles can have a substantial influence and must be addressed (e.g. see Thurston et al., 1992). Secondly, such autocorrelation violates an underlying assumption of regression time-series models (i.e., the independence of observations) (Zar, 1984), potentially yielding biased regression coefficients and coefficient significance levels for this reason. It is difficult to interpret time-series studies that fail to directly address autocorrelation in their analysis and documentation.

One method to assess first order autocorrelation in a model is the Durbin-Watson (DW) statistic provided by most standard statistical packages (Durbin and Watson, 1951; Draper and Smith, 1981). When there is absolutely no first order autocorrelation (i.e. each observation is independent from the next) in the residuals of a model (the differences between the model predicted outcome and the observed), then the model $DW=2.0$. When sequential observations of the model residuals are positively autocorrelated, then the DW statistic is less than 2.0, while negative autocorrelation results in DW values greater than 2.0. If the model DW statistic differs significantly from 2.0, then autocorrelation in the data must be addressed. More extensive insight into autocorrelation in a model can be obtained

by calculating the autocorrelation function (ACF) of the model residuals, which also provides higher order (i.e. multi-day lag) autocorrelation information.

Autocorrelation in model residuals has been addressed in recent analyses by various methods. The primary goal in invoking such measures is to eliminate the long wave "noise" in the data without removing any pollution-related health effects "signal" in the process. One method is to remove autocorrelation from the outcome variable (e.g. mortality) prior to the regression (e.g. via long wave prefiltering, see Shumway, 1988, or via prewhitening, see Ito et al., 1993). When prefiltering is employed, the dependent variables (e.g. pollution) are often also prefiltered in order to prevent long wave cycles from obscuring the acute associations being modeled, and to help clarify the lag structure in the cross-correlation exploratory analyses. Another approach is to include dependent variables in the regression which account for long wave cycles in the outcome data (e.g. by the inclusion of annual and seasonal cycle sine/cosine terms, see Thurston et al., 1992 or Schwartz, 1993). In either case, any information about long wave pollution-health associations are lost in the process of removing autocorrelation by these methods, so any pollution effects estimated are acute only, and do not include the possible chronic effects of pollution exposures. In older time-series analyses (e.g. Shimmel, 1978), autocorrelation was reduced by first regressing the outcome variable on multiple time averages and lags of other environmental variables (e.g. temperature). However, if any of these multiple "control" variables are correlated with short wave variations in pollution, this latter approach may have the serious drawback of over specifying the model and removing a short wave pollution-health effects signal of interest before the analysis has even begun. Similarly, the inclusion of autoregressive terms to remove all autocorrelation, while very conservative, also carries the risk of removing short wave associations of interest at the same time. It is important to remember that, while there are standard diagnostics available to determine whether autocorrelation remains a significant problem, no such check exists to determine whether the more severe autocorrelation removal methods (e.g. prewhitening and autoregressive modeling) may have also inadvertently removed a pollution-health effects association of interest.

Figure 1 displays time-series plots of several environmental variables measured in New York City during the 1970's. These data are a portion of a larger data set which has been previously analyzed in detail (Shimmel, 1978 and Kinney and Ozkaynak, 1992). The seasonality of all these environmental variables is obvious, with the highest respiratory mortality occurring in winter, when temperature and ozone are at a minimum and Coefficient of Haze (COH, an index of carbonaceous particle mass) is at a maximum. The influence of these trends on the raw correlations of each environmental variable with

respiratory mortality (as noted in each plot) is obvious, but potentially misleading. For example, since the average respiratory mortality is lowest when O₃ is generally high (i.e. the summer), the raw O₃-mortality correlation ($r=-0.09$, $p=0.001$) would suggest that O₃ exposure is "good" for people. This cannot be the case from a biological standpoint. It is clear that the apparently statistically significant negative correlation between O₃ and respiratory mortality is not causal, but is a spurious result of confounding by unaddressed seasonality.

Figure 2 displays time-series plots of the New York City data after applying one approach to addressing the long wave seasonal cycles: prefiltering the data. In this case, a 19-day moving average equivalent filter developed by Shumway (1988) was applied to the data by Kinney and Ozkaynak (1992), resulting in a data series of deviations from the fitted filter. Note that the filtered ozone data set has been limited here to the time of year that data are consistently available from year to year (April-September). Once the long wave cycle confounding is removed, the day-to-day environmental-respiratory mortality relationships can be determined, as noted in Figure 2. In that figure, it is seen that ozone now has a (more biologically plausible) significant positive correlation, whereas COH drops to non-significance once the seasonal confounding is addressed.

Figure 3 graphically displays, as an example, the effect of addressing autocorrelation on the COH-respiratory mortality relationship. In the first plot, there appears to be a very strongly significant relationship between respiratory mortality and the COH particle mass index. However, as revealed by the Durbin-Watson statistic ($DW=1.2$), this relationship is influenced by autocorrelation resulting from the fact that respiratory mortality and COH are both coincidentally highest in the winter. After filtering, the autocorrelation is no longer present ($DW=2.1$), but the previously apparent particulate matter-mortality relationship is seen to be non-significant once the long wave seasonal variations have been appropriately accounted for.

Model Specification

The appropriateness of a model's specification is also important to evaluate because the particular choices of dependent (health outcome) variables (the y's) and independent (environmental) variables (the x's) has a major effect on the model results. Of primary importance is that the health outcome variable considered (e.g. respiratory hospital admission) must be as objectively and unambiguously defined as possible. For example, designations as to the cause of death is not nearly as certain as the fact of death (MacMahon

and Pugh, 1970), and hospital admissions are better defined than are patient symptom diaries (Bates and Sizto, 1983).

The parallel consideration of a "control" disease outcome (i.e. one less likely to be influenced by air pollution) is desirable to support causality, by testing whether any associations found are merely artifactual (e.g. due to general confounding) (Hill, 1965). In the case of hospital admissions, this can simply involve the careful selection of another group of admissions, such as digestive diseases. However, identifying a control disease category for the mortality effects of environmental stresses, such as air pollution or heat stress, is not as easy as for hospital visits or admissions, since such generalized stresses may adversely affect any severely compromised person, regardless of their particular illness.

Environmental variables should be representative of (i.e. correlated with) population exposures over time. Absolute bias in exposure measurements is not as important in time-series analyses as in cross-sectional analyses, since the comparisons are over time at a single locale, not between the average concentrations at various sites. However, random measurement errors which reduce the correlation between measured and actual exposure will reduce the significance of the pollutant effect and may bias pollutant effect regression estimates toward zero (Thomas, Stram, and Dwyer, 1993). As shown in a companion paper in this volume, the strength of pollutant time series associations with health effects can vary dramatically from monitoring site to monitoring site within an airshed (Ito et al., 1994), so the representativeness of monitoring sites employed in a health effects analysis should be confirmed before the analysis begins.

In building a model, it is important not to over specify or under specify the model. For example, the analysis should consider as many copollutants as possible, so that the effects of an excluded pollutant are not incorrectly ascribed to a correlated, but not causal, pollutant that is included in the model. Conversely, it is important to recognize that simultaneously including environmental variables which are highly correlated with other dependent variables in the model can yield biased pollutant coefficients. For example, the inverse of wind speed ($1/u$), an excellent general index of pollution dispersion which is itself unlikely to be a directly causal threat to health, would be inappropriate to include simultaneously with pollution concentrations, as this would serve only to obscure the individual pollutant's effect. The importance of such intercorrelations among a model's "independent" variables should be assessed by calculating and reporting model coefficient intercorrelations (r_b). If two biologically plausible dependent variables' coefficients have a significant intercorrelation when entered simultaneously, then the modeler should report the model with and without each variable in order to assess the range of possible associations attributable to each variable.

Potential lags between cause and effect must also be addressed. Exploratory cross correlation analyses between dependent and independent variables should be conducted prior to the modeling in order to identify any such lags and to allow them to be considered when specifying the model. However, misleading cross-correlation results can arise if autocorrelation exists in either or both of the variables being cross correlated, so it may be necessary to prefilter each of the series before conducting the cross correlation. Moreover, if pollution exposure is correlated with effects on the same day as (or days following) exposure, but not before, the model temporality is consistent with causality (Hill, 1965).

Figure 4 displays an example exploratory cross correlation analysis of a previously reported hospital admissions-air pollution data set from Toronto, Ontario (Thurston et al., 1994). In this analysis, the temporality of the cross correlations is consistent with causality, since all particulate matter correlations with respiratory admissions are for the same day as exposure or for the day following exposure. Also, the lack of significant correlations ($r > 0.20$), or even a pattern of the weak correlations ($0.15 < r < 0.20$), between the pollution variables and the control admissions is also supportive of causality. It is interesting to note that, had only one of Total Suspended Particulate Matter (TSP), Particulate Matter less than 10 mm in aerodynamic diameter (PM10), or Fine Particulate Matter less than 2.5 mm in aerodynamic diameter (FP) been considered in the analysis, that metric would have appeared to be the particulate metric of relevance to human health. However, by considering them all individually, and by examining the TSP-PM10 and the CP (Coarse Particulate Matter, which equals PM10-FP) correlations, it became clear that the FP portion of the mass (including the particulate strong acidity, H^+) is driving the apparent relationships seen for the PM10 and the TSP metrics of particulate matter. Thus, a thorough exploratory cross correlation analysis can reveal a great deal about the variables' interrelationships, providing guidance for the subsequent model specification process.

Data Distribution Effects

The distribution of the dependent variable in the analysis must also be considered when choosing the model design. For example, infrequent events, such as respiratory mortality and hospital admission, have non-normal underlying distributions over time. This can lead to non-normal model residuals with heterogeneous variance, which are violations of the underlying assumptions of many time-series models. Such "rare event" distribution skewness is most problematic below an average of about 4 counts per day, when the deviation from normality is greatest (Armitage, 1971).

Data distribution problems are usually dealt with in either of two ways. The first approach is to use aggregated counts (e.g., admissions/day in an entire metropolitan area),

rather than counts from, for example, a single hospital in a city. Since the sum of non-normal distributions tends toward normality, count aggregation diminishes this concern, and ordinary least squares (OLS) modeling (i.e. regression) methods can usually be safely applied. Furthermore, heterogeneity of residual variance, if present, can be addressed via weighted least squares modeling. It is important that the model residuals be checked for normality (via the W statistic) to assess whether the model chosen has appropriately accounted for distributional deviations from normality. The other approach to address this problem is to use smaller subsets of data (e.g., admissions/day at a hospital), but employ modeling methods designed to handle such data. These methods have included, for example, Poisson models (in which the mean of the outcome variable equals its variance), or Generalized Estimating Equation (GEE) models, when the variance changes with y , but does not equal the mean (Schwartz, 1993).

EXAMPLE MORTALITY MODELING RESULTS

Table 1 shows the results of selected example time-series regressions of the 1972-1975 New York City data shown in Figures 1 and 2. Model 1 is a "naive" case, in which the unfiltered respiratory mortality is regressed via OLS on the three unfiltered environmental variables. All the variables are apparently significant, though the ozone coefficient is negative (contrary to biological plausibility). However, the DW and W statistics shown in this table indicate that there is a serious autocorrelation problem for Model 1 ($DW \ll 2$) and that this model's residuals are significantly different from normal. The second model has limited the analysis to the O₃ season (April-September), which improves these diagnostics quite a bit, but the model still has a lot of positive autocorrelation (due to the within-season influences of the annual cycle). Using the filtered data from Figure 2, Model 3 yields results with satisfactory DW and W statistics, and indicates conclusions very different from the original "naive" model. For example, the O₃ term is now significant and in the (biologically plausible) positive direction.

As an alternative to the use of prefiltering, Models 4 and 5 in Table 1 consider the use of multiple sine and cosine waves (e.g. the 1 mo., 3 mo., 6 mo. cycles, etc.) in OLS and a Poisson models, respectively. Though the autocorrelation is not as completely removed, and though the Poisson model does give non-normal residuals, these two models yield very similar results to the filtered OLS model. The similarity of the Poisson and OLS results are no doubt in part due to the fact that the New York City daily mortality counts are aggregated over a large population, and in part due to the filtering out of the seasonal cycles, both of which decrease skewness in the data distribution. Also, these aggregated mortality

data do not exhibit an increase in variance at higher mortalities (i.e. in the winter), as would be expected in a Poisson distributed variable. Thus, while OLS and Poisson models can give quite different results if the counts per day are extremely low, or if the variance of the model residuals increase noticeably with the magnitude of the effect, it is not surprising that the OLS and Poisson results are similar in situations like the one considered here.

Although not presented in Table 1, a limited sensitivity analysis of the effect of model specification on those results was also conducted. In particular, daily Relative Humidity (RH) was added to Model 3. The diagnostics were virtually unchanged, but the COH and O₃ coefficients and their significances were slightly modified. The COH coefficient p-value was still clearly non-significant, but the O₃ coefficient decreased and became marginally non-significant (one-way $p=0.06$). The reason for this was revealed by examining the intercorrelations of the coefficients ($r\beta$) of this model, which revealed that the RH coefficient was significantly and negatively correlated with O₃ ($r\beta=-0.20$). As a result, the addition of an RH term was partially at the expense of the O₃ coefficient in the model. Ultimately, the modeler must attempt to interpret such variations in results with changes in model specification vis-a-vis the biological plausibility of each term. This limited sensitivity analysis exemplifies the importance of both considering various model specifications and of examining model diagnostics in the conduct of such modeling.

CONCLUSIONS

In time-series analysis no model is perfect, but some models are more wrong than others. Clearly, the model *can* become the message, since the conclusions resulting from alternative models can vary dramatically, especially if the underlying model assumptions have been violated. The modeling factors identified in this paper as critical to be evaluated are: autocorrelation of the model residuals; the model specification, and; the distribution characteristics of model residuals.

While no specific prescription for data analysis can be given for all situations, some general guidelines are suggested by the above discussions and examples. An effort should be made to include as many potentially confounding variables as possible (e.g. other pollutants) in the analysis. However, the inclusion of non-biologically plausible covariables which are correlated with pollution in general (e.g. inverse wind speed) should be avoided. Pollutant measurements employed should be as representative of (i.e. correlated with) population exposures as possible. When available, a non-respiratory health outcome should be evaluated as a "control". Exploratory statistical analyses should be conducted

prior to the statistical modeling, potentially including: the intercorrelations of the x's; variable cross-correlations (i.e. y's on x's), and; autocorrelation functions for all y's and x's. Also, autocorrelation in model residuals should be evaluated (e.g. via the DW statistic). If present, autocorrelation should be addressed by appropriate methods, possibly including: an analysis of the data by season, data prefiltering, the inclusion of model terms for all possible long-wave cycles, and/or autoregressive methods. Intercorrelations among the coefficients of model variables (r_{β}) should also be evaluated to aid in the interpretation of model results. Furthermore, OLS model residuals should be checked for deviations from normality (W), and, if present, non-normalities should be addressed (e.g., via Poisson modeling for count data). Furthermore, given the previously noted potential variations in results with differing modeling approaches, it is desirable for such analyses to include a sensitivity analysis inter comparing various models and model specifications.

If the above noted time-series modeling suggestions are more consistently implemented and reported in future published analyses, better models may or may not result. However, the ease of evaluating and inter comparing results from such time-series epidemiologic studies would surely be improved. For example, this would aid efforts to employ time-series analyses in the air pollution standard setting process (e.g. in the development of pollutant Criteria documents).

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TABLE 1. EXAMPLE COMPARISONS OF UNFILTERED VS. FILTERED VS. POISSON TIME SERIES REGRESSIONS
OF 1972-1975 NEW YORK CITY RESPIRATORY MORTALITY

Model	COH Coeff. (p-value)*	O ₃ Coeff. (p-value)*	T Coeff. (p-value)*	DW Stat	W Stat (p-value)
1. Unfiltered OLS (year-round)	2.6 ± 0.4 (p = 0.0001)	20.9 ± 4.8 (p = 0.0001)	-0.09 ± 0.01 (p = 0.0001)	1.39	0.971 (p = 0.0001)
2. Unfiltered OLS (O ₃ season)	2.2 ± 0.5 (p = 0.0001)	14.3 ± 4.9 (p = 0.002)	0.02 ± 0.02 (p = 0.10)	1.63	0.983 (p = 0.22)
3. 19-day Moving Avg. Filtered OLS (O ₃ season)	-0.3 ± 0.7 (p = 0.48)	10.7 ± 5.0 (p = 0.02)	0.06 ± 0.03 (p = 0.02)	2.06	0.984 (p = 0.34)
4. OLS with 10 Sine/Cosine Waves (O ₃ season)	0.2 ± 0.7 (p = 0.39)	12.5 ± 5.0 (p = 0.005)	0.09 ± 0.03 (p = 0.0002)	1.75	0.984 (p = 0.28)
5. Poisson with 10 Sine/Cosine Waves (O ₃ season)	0.03 ± 0.8 (p = 0.48)	11.8 ± 5.5 (p = 0.02)	0.13 ± 0.04 (p = 0.0003)	1.83	0.975 (p = 0.0005)

* one-way test.

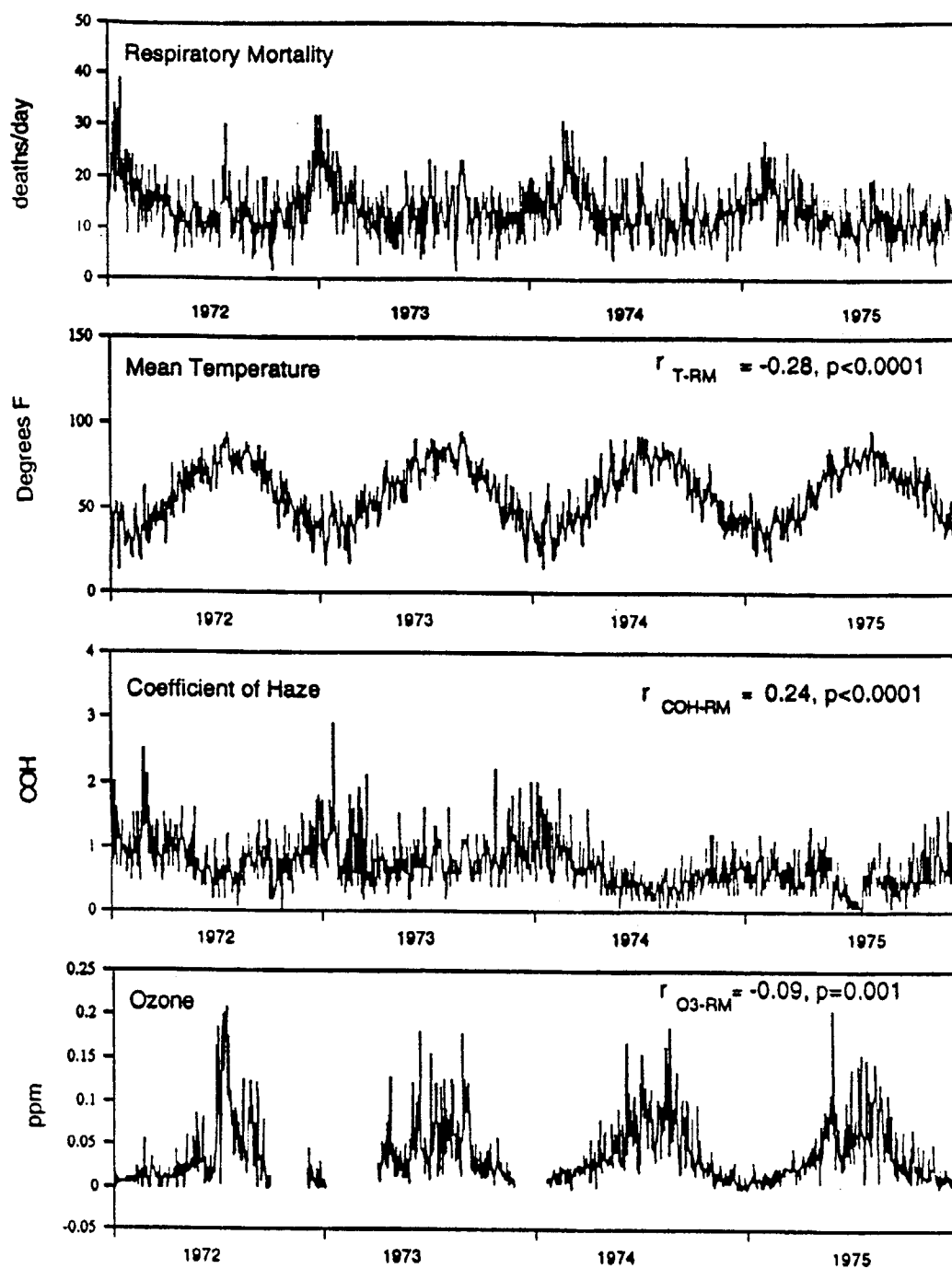


Figure 1. Unfiltered Daily Respiratory Mortality and Environmental Data from New York City (1972-1975).

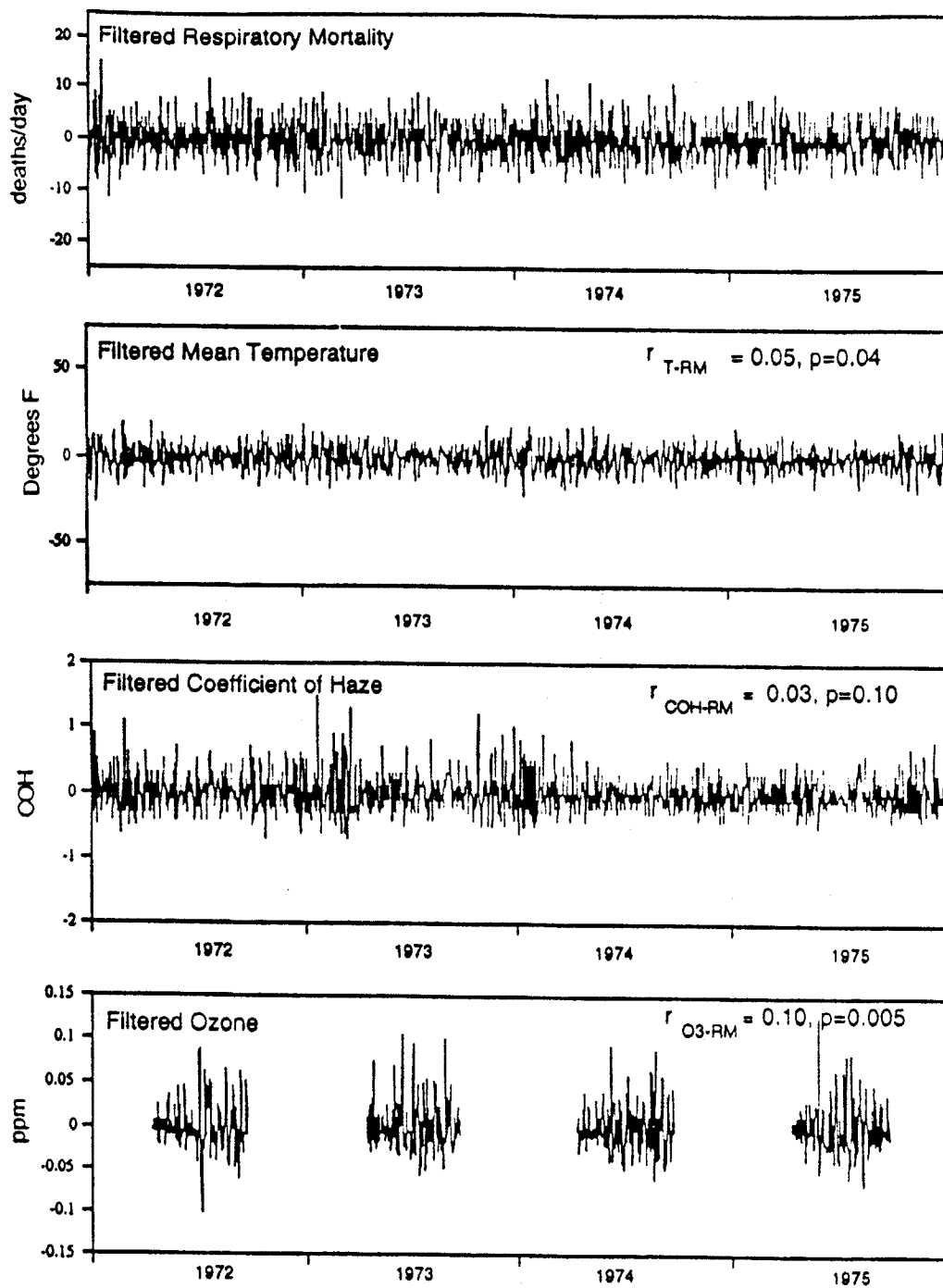


Figure 2. Filtered Daily Respiratory Mortality and Environmental Data from New York City (1972-1975).

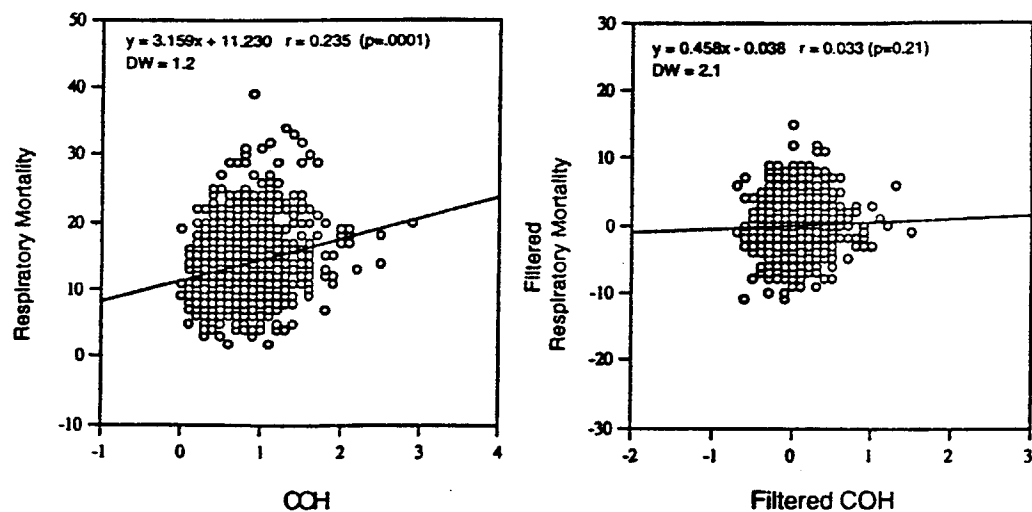


Figure 3. Comparison of the New York City Coefficient of Haze (COH) Relationship with Respiratory Mortality, Before (unfiltered) and After (filtered) Removing Autocorrelation.

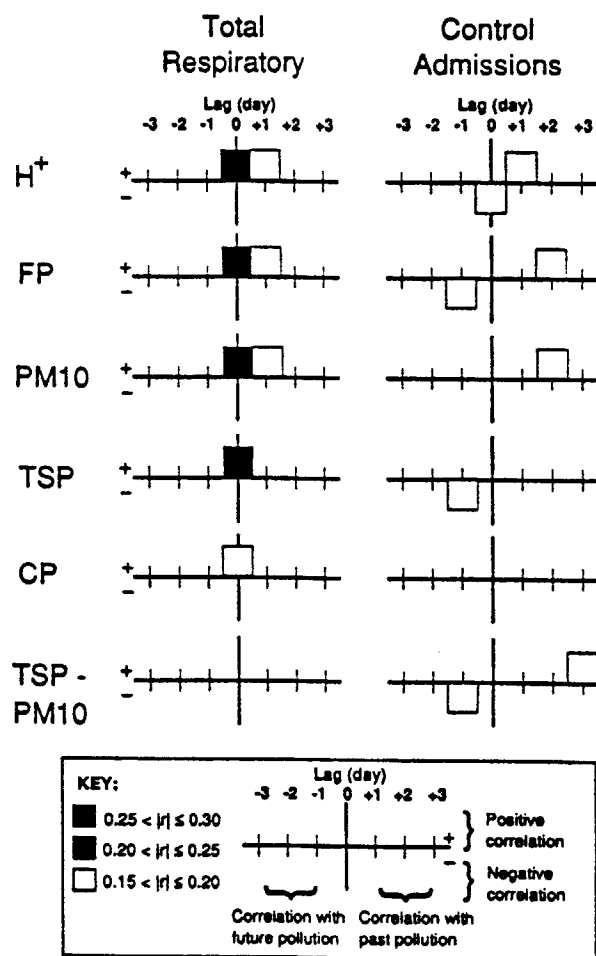


Figure 4. Crosscorrelations between Daily Hospital Admissions and Various Metrics of Particulate Pollution in Toronto, Ontario (Thurston et al., 1994)

SEPARATING THE EFFECTS OF TEMPERATURE AND SEASON ON DAILY
MORTALITY FROM THOSE OF AIR POLLUTION IN LONDON: 1965-1972

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ABSTRACT

Most analyses of the large data base of daily mortality and indices of pollution in London, England have dealt with the confounding influence of ambient temperature and/or season by using empirical adjustment models in the determination of the regression coefficients for the pollutants. The conclusions about the influence of the measured pollutants, i.e. aerosol strong acid (H^+), sulfur dioxide (SO_2), and black smoke (BS), on mortality have varied due, at least in part, to the selection of the form of the temperature/season adjustment model. We have taken an alternate approach to separate the influences of temperature, season, and ambient pollutant levels on daily mortality in Greater London between 1965 and 1972. In each season, the majority of days fell within one or two temperature ranges, within which the daily death rates also fell within narrow ranges. Within these restricted temperature and mortality ranges, preliminary analyses indicated that there were relatively strong associations between daily mortality and the daily logs of the concentrations of H^+ and SO_2 that were not confounded by temperature or seasonal variations. By contrast, the associations between the daily log of BS and daily mortality in these restricted ranges were weaker, especially in the winter and summer seasons. While a more comprehensive analysis of these London data and of other pollutant and mortality data sets is needed, these initial results suggest that this new approach can serve as a valuable complement to model-based approaches for studying associations between pollutant exposures and daily mortality.

INTRODUCTION

Many analyses of the influence of air pollution on daily mortality in humans have taken advantage of the availability of a very large set of daily mortality data for Greater London and temporally coincident data on temperature (T), humidity, black smoke (BS) and sulfur dioxide (SO₂). Daily data for winters became available in 1958, and year-round data became available in 1965. The environmental data were collected at seven sites in Greater London, and most analyses of associations between the environmental variables and mortality have utilized the average values for these seven sites.

All of the time-series studies using these London data sets have found associations between daily mortality and temperature and, after adjustment for temperature, day-of-week, and season, have found residual associations of daily mortality with BS as well (Mazumdar et al., 1982; Shumway et al., 1983; Roth et al., 1986; and Schwartz and Marcus, 1990). However, they differed in their conclusions about the extent of association of daily mortality with SO₂. In multiple regression analyses, Mazumdar et al. found no independent association of daily mortality with SO₂; Schwartz and Marcus found only a small role for SO₂; Shumway found that SO₂ and PM were equally predictive; and Roth et al. concluded that it was not possible to separate the influence of BS and SO₂. We attribute these differences in results, in part, to the differences in the nature of the methods used to account for the longer-wave phenomena in the time-series that affect daily mortality. This dependence of the conclusions on the model used is discussed in greater detail by Thurston and Kinney (this Colloquium).

Between 1965 and 1972, concurrent continuous daily records of mortality were available for correlation with daily measurements of aerosol strong acid (H⁺) that were made at a central London site (St. Bartholomew's Medical School). In a preliminary, time-series analysis of the data set, we had indicated that H⁺ appeared to be more strongly associated with total daily mortality than either BS or SO₂ (Thurston et al., 1989). However, a more detailed analysis of the same data set by Ito et al. (1993), involving "pre-whitening," did not provide support of our hypothesis that H⁺ would have a greater degree of association with daily mortality than BS or SO₂ (Ito et al., 1993). In the Ito et al. (1993)

analysis we found that temperature had the greatest influence in all seasons, that all three of the pollution variables (same day and lagged one or two days) were significantly associated with daily mortality, and that it was not possible to assign a dominant role to any one of them because the temporal fluctuations of the pollutants were highly collinear. Table 1 shows results from this earlier study that show the apparent correlation coefficients among the pollutants measured at the central site (St. Barts) and the average values of BS and SO₂ measured concurrently at the seven station Greater London network. In the course of the Ito et al. (1993) analysis, we also discussed the limitations imposed on the analysis by the use of only one monitoring site, the limited precision of the measurement of H⁺, and the optimal selection of filters for controlling the confounding long-wave influences.

It can be seen in Table 1 that H⁺ was more strongly correlated with SO₂ at the central site ($r=0.77$) than with the seven-site mean SO₂ ($r=0.71$) and with the central site BS ($r=0.68$) than seven-site mean BS ($r=0.61$). While we recognize that Greater London daily mortality is somewhat more highly correlated with seven-site BS and SO₂ than with central site levels, we have restricted our analyses of associations in this paper to the central site where H⁺ data were available along with BS and SO₂.

While pollution and mortality tend to rise and fall together, mortality and temperature tend to follow an inverse relationship. In this paper, we describe and apply an alternate approach to the separation of the effects of temperature and pollution on daily mortality; one that does not rely on adjustments to the raw data, but rather on the selective use of major subsets of the data for which seasonal and ambient temperature fluctuations are not significant confounding factors.

METHODS

Initial Data Sets Used

All of the data sets used in these analyses were subsets of the data that have been analyzed previously, and have been described in detail (Thurston et al., 1989; Ito et al., 1993). All the pollution variables used were those measured at the Medical College of St. Bartholomew's Hospital (Commings and Waller, 1967), and the 9 am temperature was measured at the London Meteorological Office, which was located close to the Medical

College. Summaries of these data are displayed in Figure 1 for H^+ , SO_2 , BS, and T. In each panel, each point represents the mean and standard error of 20 days of the overall data set as sorted according to the values of the abscissa variable, following the example used by Schwartz and Marcus (1990). The ordinate is the average value for the raw data for daily total mortality in Greater London.

Separation into Seasonal Data Sets

Our first step in the analysis was to separate the data by season. For winter, we chose the traditional winter interval used by all previous investigators of London daily mortality, i.e., the calendar months of November, December, January, and February (903 days). For summer, we selected May, June, July, and August (984 days). We then consolidated the remaining spring and fall data, i.e., March, April, September, and October (945 days). Summaries of these data sets are displayed in Figure 2. As in Figure 1, each point represents the mean and standard error for 20 days, as sorted by the values of the respective abscissa variable.

Selection of Restricted Ambient Temperature Ranges

By visual examination of the mortality vs. temperature plots in Figure 2, one can see that there are either one or two ranges of temperature in each season over which the average daily mortality is relatively constant. In the winter, there is one such period, in the mid-range, and there is more substantial variation in daily mortality occurring at both the upper and lower bounds of temperature. The same is true for the summer. For the spring/fall data, the greatest change in average daily mortality with temperature occurs in the mid range of temperature. For these data, we were able to select two temperature ranges over which mortality variations were judged to be minimal. For winter, we chose the temperature range of 5-10°C, for which there were 461 days, having a mean daily mortality of 272, and a standard deviation of 42. For summer, we selected the temperature range of 13-18°C, for which there were 610 days, with a mortality of 205 ± 19 . For the spring/fall data set, we selected two ranges of temperature, i.e., 4-9°C and $> 12^\circ\text{C}$. For the lower band of temperature, there were 275 days with a mortality of 253 ± 27 , while for the days $> 12^\circ$, there were 418 days, with a mortality of 217 ± 22 .

RESULTS

In the following results, the correlation coefficients shown in Table 2 are those for the 20-value-averaged points, to indicate the fit of lines. Since the averaging necessarily smoothes the “noise”, these correlations are higher than the correlation for all of the individual points, which are presented in Table 3, along with the correlations of the environmental variables for the daily data.

Winter Data ($5^{\circ} \leq T \leq 10^{\circ}$): The data for the days with temperatures between 5 and 10°C ($41\text{-}50^{\circ}\text{F}$) are displayed in Figure 3. For H^{+} , there is a strong correlation between the log of the ambient concentration, expressed as sulfuric acid, and total daily mortality, with $r = 0.75$ for the points representing 20 day averages (Table 2) and 0.26 for the individual days results (Table 3). By contrast, there is only a very weak association between the log of BS and daily mortality, with $r = 0.12$ (20 day) and -0.04 (individual day). For SO_2 , there was an intermediate degree of association, with $r = 0.45$ (20 day) and 0.08 (individual day).

Summer Data ($13^{\circ} \leq T \leq 18^{\circ}$): The data for the days with temperatures between 13 and 18°C ($55.4\text{-}64.4^{\circ}\text{F}$) are displayed in Figure 4. Once again, there is a correlation between the log of the ambient concentration of H^{+} (as H_2SO_4) and daily mortality, with $r = 0.67$ (20 day) and 0.14 (individual day). The association between the log of BS and daily mortality, with $r = 0.41$ (20 day) and 0.04 (individual day), was weaker. For SO_2 , the association was strongest, with $r = 0.82$ (20 day) and 0.24 (individual day).

Spring/Fall Data ($4 \leq T \leq 9^{\circ}$): For these cooler spring/fall days, the data displayed in Figure 5 show that there are strong correlations between the log of H^{+} and mortality, with $r = 0.88$ (20 day) and $r = 0.30$ (individual day), and the log of SO_2 and mortality, with $r = 0.92$ (20 day) and $r = 0.29$ (individual day). For the log of BS, in this dataset, the correlation with mortality was almost as strong as for H^{+} and SO_2 , with $r = 0.84$ (20 day) and 0.19 (individual day).

Spring/Fall Data ($T > 12^{\circ}\text{C}$): For these warmer spring/fall days, the data displayed in Figure 6 show, once again, correlations between the log of H_2SO_4 and mortality, with $r = 0.77$ (20 day) and $r = 0.14$ (individual day), and the log of SO_2 and mortality, with $r = 0.85$ (20

day) and $r = 0.32$ (individual day). The extent of correlation for BS with mortality was somewhat lower, with $r = 0.69$ (20 day) and $r = 0.07$ (individual day).

DISCUSSION AND CONCLUSIONS

The very large sizes of the overall data sets available for this study has permitted segregation of the pollution and mortality data into subsets by seasons, and within season by temperature, producing temperature subsets for each season that were still large enough for examination of strength of association between pollution and mortality. It is unlikely that other potential confounders in time-series analyses, such as day of week, variations in cigarette smoking, type of heating system, etc., are of any greater or lesser consequence within limited ranges of temperature and mortality used as the basis for editing the larger data sets. By using the data from each season separately, we have reduced confounding that accounts for large variations in daily mortality from season to season. Likewise, by editing the data by temperature, we have greatly reduced confounding by the single strongest confounding factor in all time-series studies of such data.

The results of this exploratory analysis of a new approach to the study of the possible effects of air pollutants on daily mortality are far from definitive. They seem to show that the method is worthy of further development and application, if only on the basis that strong associations between pollutant concentrations and total mortality remain after virtually all of the influences of season and temperature are separated out by careful selection of data subsets containing minimal confounding by these variables.

In this preliminary analysis, we made some arbitrary selections of variables on the basis of their utility in earlier studies. For example, we used only same day mortality, and we did most analyses using pollutant and mortality values averaged over 20 days as sorted by the pollutant variable. We also presented our regressions and correlations on the basis of the logs of concentration on the basis of their better fit than the linearized pollutant variables. If the lagged relationships with mortality are different for the different pollutants, or if other curve fitting paradigms are more appropriate, then any comparisons drawn on the basis of the regression slopes and correlation coefficients presented in this paper may be misleading.

With the caveats implicit in the preceding discussion, the results of these preliminary analyses, as summarized in Tables 2 and 3, raise interesting questions. Among these are:

1) What accounts for the lesser strength of association between BS and mortality in comparison between H^+ and SO_2 and mortality? The associations for BS seem even weaker in Table 3, for the individual days' data, than in Table 2 for the data grouped in blocks of 20 days. These results were surprising, since most previous studies have found BS to be at least as strong or a stronger predictor of mortality as SO_2 . Also, the mass concentrations of fine particles, i.e., particles $< 2.5 \mu m$ in aerodynamic diameter (FP), particles $< 10 \mu m$ in aerodynamic diameter (PM_{10}), and total suspended particulate matter (TSP), have been shown to be significantly associated with human mortality and morbidity in a variety of communities (Pope, et al., 1994), including some with little or no measurable aerosol H^+ . This suggests that: 1) the opacity of the particles may be a less relevant index of exposure than aerosol mass concentration; 2) the measurements or estimates of aerosol H^+ were not sufficiently sensitive or reliable; or 3) an unknown artifact or confounding factor may account for the health effects that have been associated with aerosol mass.

2) Does H^+ really have a greater influence on mortality in the winter than SO_2 , and a much greater influence role than BS? The correlation between H^+ and BS in the winter ($r = 0.27$) is much lower than any other seasonal cross correlation of pollutant pairs, and the pollutant-mortality correlation results are in the same order for both the 20 day averages (Table 2) and the individual day data (Table 3).

3) Does SO_2 really have a greater influence on mortality in the summer than H^+ , and an even greater influence than BS? Here also, the results are in the same order for the 20 day averages and the individual day data. However, a causal role for SO_2 alone seems unlikely on the basis of the analyses of Buechley (1973). In that analysis, the strength of association between SO_2 and mortality in the New York metropolitan region in 1963 and 1973 was similar for time periods a decade apart, despite the drop in average levels of SO_2 in the air by a factor of ten in the intervening years.

4) If BS is not causally associated with excess daily mortality, but H^+ and SO_2 are, does BS gain strength as an indicator by being time-averaged? BS appears to have a stronger association with mortality in this study when averaged over 20 days than when the individual days' data are used. Other daily mortality studies have utilized 15-day moving averages or other smoothing functions. In such studies, it may be the high degree of intercorrelation of BS with SO_2 that then allows BS to serve as a useful indicator of pollution stress.

If, in fact, it was H^+ that was the important causal factor for the excess mortality in London air pollution, then it has major implications for H^+ exposures in other times and places. Less than 5% of the summertime days in the temperature edited data set had H^+ concentrations $> 5 \mu\text{g}/\text{m}^3$ (as H_2SO_4), and the average was $2.6 \mu\text{g}/\text{m}^3$. By comparison summertime average H^+ concentrations in Albany, Buffalo, and White Plains, NY in 1988 were 2.5, 2.2, and 2.4, while for Toronto, Ontario in 1988 it was $2.6 \mu\text{g}/\text{m}^3$ (as H_2SO_4). Thus, the strong association seen between H^+ in London and daily mortality have relevance to exposures that commonly occur over most of the eastern U.S. and lower Ontario each summer.

The graphically-based analytic approach used for these preliminary analyses of the 1965-1972 London data sets will, in the future, be evaluated in greater detail. It will also be applied to the New York State and Toronto daily mortality and H^+ , SO_2 and PM_{10} data sets described by Thurston et al. (1992, 1994) to evaluate whether this new approach has more general utility for defining the influence of air pollution on daily mortality.

In summary, this exploratory technique permits a separation of several factors that affect daily mortality, i.e., temperature and season, from pollutant exposure factors. Further refinements in this analytical approach need to be made in terms of criteria for selecting the data subsets for analysis, for grouping of individual days' data into appropriate sized groups, for taking lagged effects into account, and for considering analytical uncertainties in exposure assessment. The results of preliminary analyses presented here raise new and interesting questions about the relative roles of H^+ , SO_2 , and BS in variations of daily mortality among large populations. These questions warrant further analyses.

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Table 1. Apparent Correlation Coefficients Among the Pollution Variables for 1965-1972
Consecutive Daily Data in London, England

	7_BS	c_BS	7_SO ₂	c_SO ₂
c_BS	0.786			
7_SO ₂	0.848	0.776		
c_SO ₂	0.707	0.800	0.792	
c_H ⁺	0.613	0.682	0.707	0.765

c_: Central London site (St. Bart's); 7_: mean of 7 sites in Greater London.

Table 2. Summary of Associations Between Pollutants and Total Daily Mortality in London, 1965-1972 in Each Season for Days with Minimal Confounding of Mortality by Temperature

Season	Regression Slopes of Mortality vs. Log Concentration ^a (± S.E.)			Correlation of Mortality with Log Concentration ^a		
	H ⁺	SO ₂	BS	H ⁺	SO ₂	BS
Winter (5-10°C)	43.6±(8.4)	30.3±(13.2)	6.2±(11.1)	0.750	0.447	0.121
S/F (4-9°C)	39.0±(5.9)	46.1±(5.8)	32.8±(6.1)	0.884	0.916	0.840
S/F (> 12°C)	21.3±(4.1)	32.4±(4.7)	15.6±(3.8)	0.765	0.847	0.685
Summer (13-18°C)	15.2±(3.1)	26.3±(3.4)	9.4±(3.9)	0.670	0.818	0.413

^a For points plotted in Figures 3-6, where each point represents the average of 20 days sorted by the abscissa variable.

H⁺ represents the concentration of strong acid aerosol expressed as H₂SO₄ in µg/m³.

SO₂ is the concentration of sulfur dioxide reported in µg/m³.

BS is the concentration of black smoke reported in µg/m³.

T is the 9 AM temperature measured at the London Meteorological office.

Table 3. Summary of Correlation Coefficients (r) for Individual Days' Data in Each Season for Days within Temperature Ranges having Minimal Confounding of Mortality by Temperature

Season	Correlation of Mortality with Log Concentration			Correlations Among Environmental Variables				
	H ⁺	SO ₂	BS	H ⁺ :SO ₂	H ⁺ :BS	BS:SO ₂	T:H ⁺	T:SO ₂ T:BS
Winter (5-10°C)	0.255	0.079	-0.039	0.536	0.268	0.576	-0.02	0.07 -0.13
S/F (4-9°C)	0.302	0.294	0.189	0.573	0.444	0.583	-0.21	0.10 0.08
S/F (> 12°C)	0.142	0.319	0.072	0.571	0.588	0.611	0.02	-0.08 0.05
Summer (13-18°C)	0.138	0.236	0.044	0.435	0.396	0.462	0.01	0.07 -0.01

FIGURE CAPTIONS

Figure 1. Plots of average daily total mortality in Greater London between April 1, 1965 and December 31, 1972 vs: a) aerosol acidity (as $\mu\text{g}/\text{m}^3$ of sulfuric acid; b) sulfur dioxide - $\mu\text{g}/\text{m}^3$; c) black smoke - expressed as $\mu\text{g}/\text{m}^3$ of dust; and d) ambient temperature - $^{\circ}\text{C}$. Each point represents the average for twenty days as sorted according to the abscissa variable. The error bars on each point show the standard deviation for the 20 measurements.

Figure 2. Plots of average daily total mortality in Greater London in different seasons. The top row is the subset of the Figure 1 data for the winter season (Nov., Dec., Jan., Feb.). The middle row is the subset for summer (May, June, July, Aug.). The bottom row is the subset for spring/fall (Mar., Apr., Sept., Oct.). Within each seasonal subset, each point represents 20 days.

Figure 3. Plots of daily average daily total mortality in Greater London vs. temperature and vs. log of pollutant concentrations for winter days with ambient temperatures between 5 and 10°C . Each point represents 20 days.

Figure 4. Plots of daily average total mortality in Greater London vs. temperature and vs. log of pollutant concentrations for summer days with ambient temperatures between 13 and 18°C . Each point represents 20 days.

Figure 5. Plots of daily average total mortality in Greater London vs. temperature and vs. log of pollutant concentrations for spring and fall days with ambient temperatures between 4 and 9°C . Each point represents 20 days.

Figure 6. Plots of daily average total mortality in Greater London vs. temperature and vs. log of pollutant concentrations for spring and fall days with ambient temperatures above 12°C . Each point represents 20 days.

All Seasons

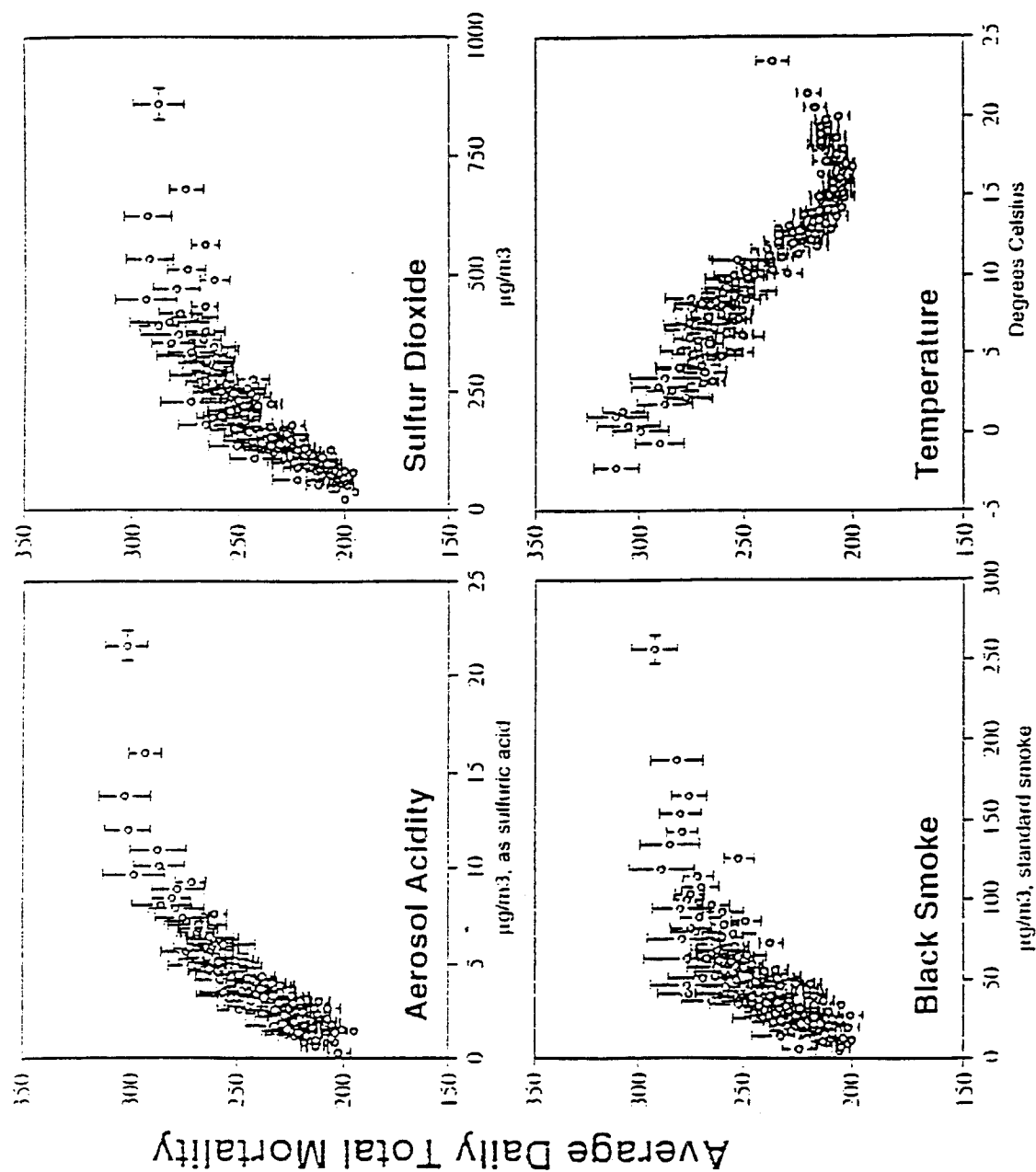


Figure 1. All Plots were produced by taking averages of 20 adjacent points that were sorted by pollution or temperature. All data: April 1, 1965-December 31, 1972 (n=2832)

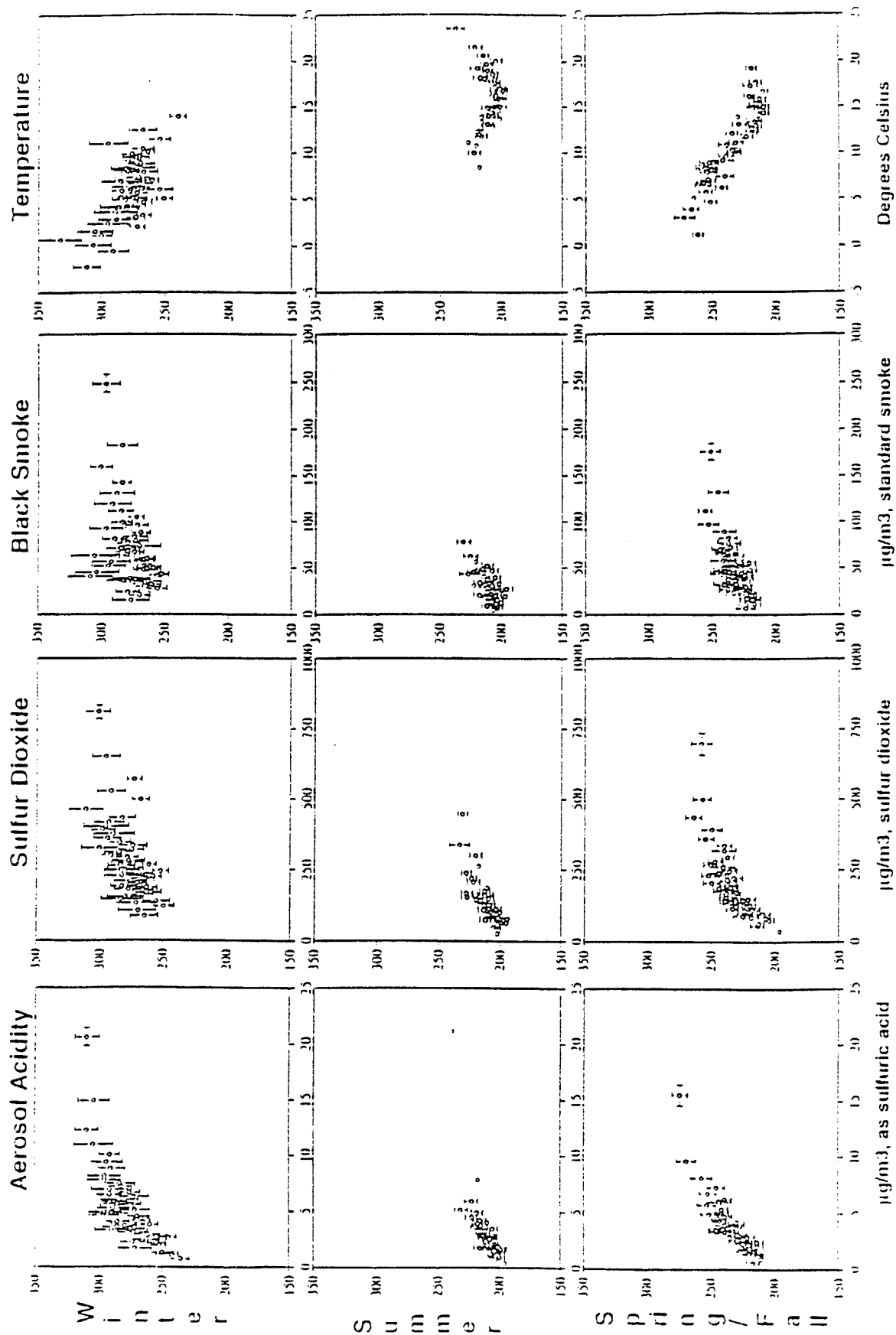


Figure 2. All Plots were produced by taking averages of 20 adjacent points that were sorted by pollution or temperature

London: 1965-1972, Winter ($5^{\circ}\text{C} \leq \text{temp.} \leq 10^{\circ}\text{C}$)

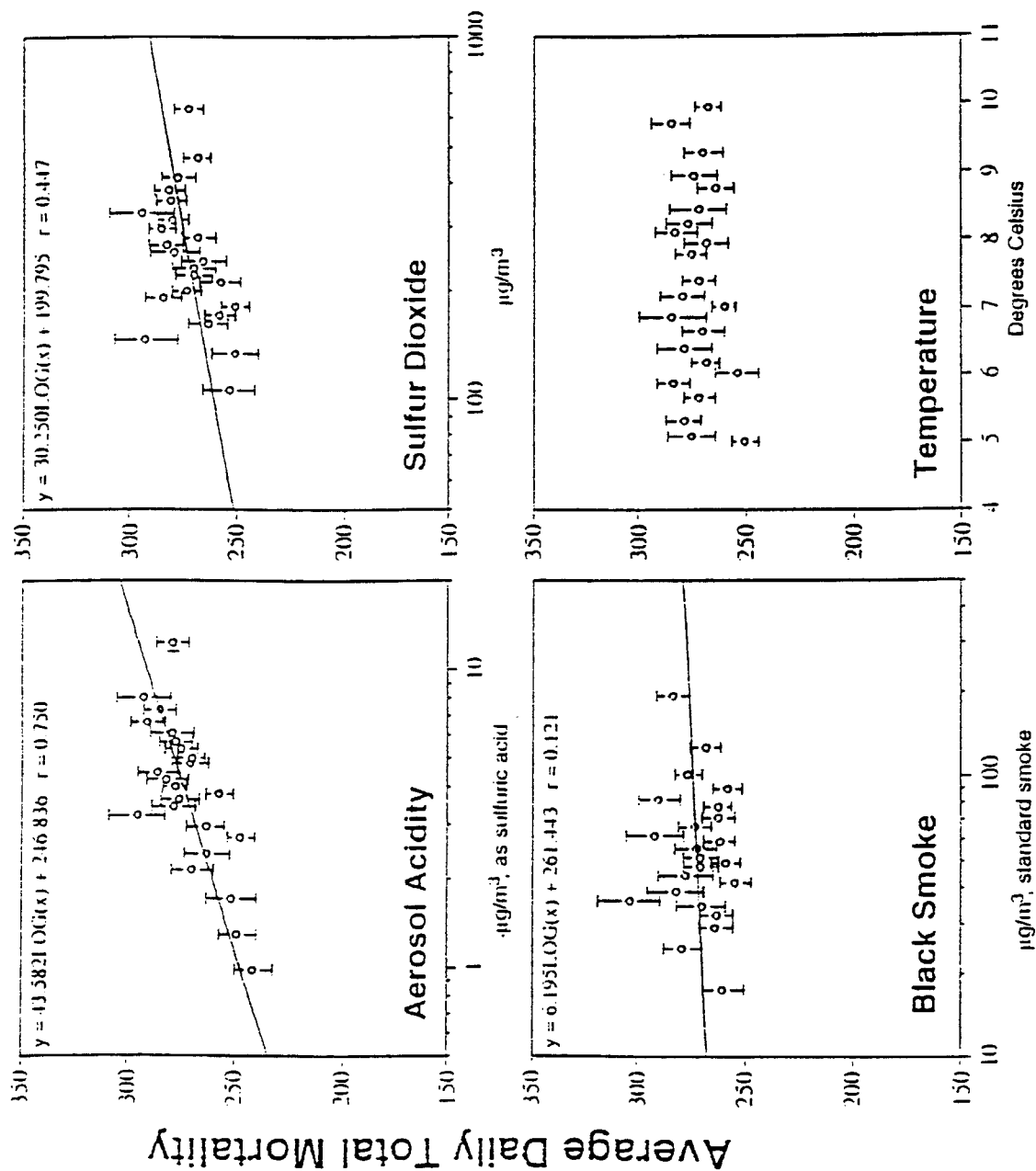


Figure 3. All Plots for winter data where $5^{\circ}\text{C} \leq \text{temperature} \leq 10^{\circ}\text{C}$ ($n=461$) were produced by taking averages of 20 adjacent points that were sorted by pollution or temperature.

London: 1965-1972, Summer ($13^{\circ}\text{C} \leq \text{temp.} \leq 18^{\circ}\text{C}$)

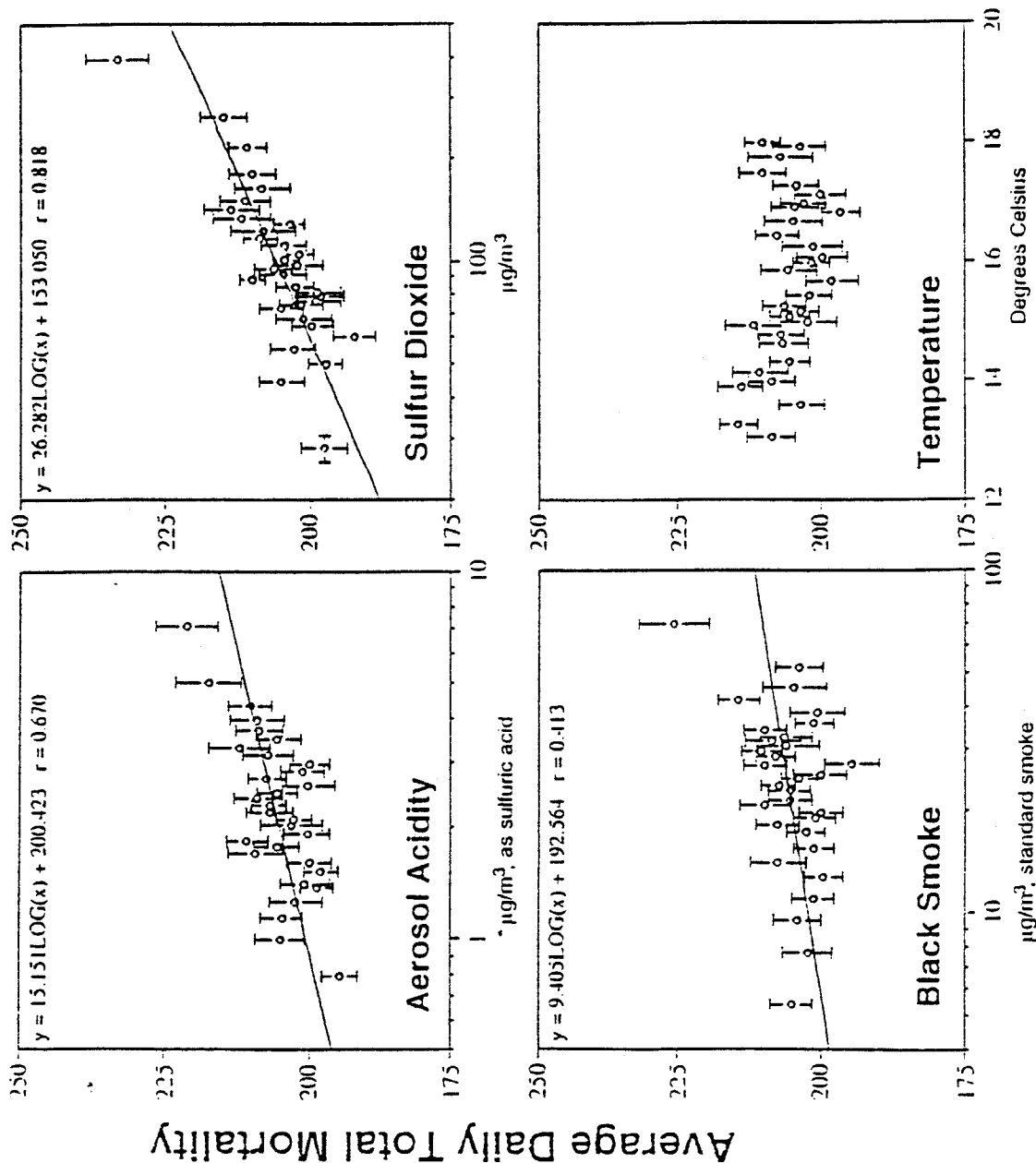


Figure 4 All Plots for summer data where $13^{\circ}\text{C} \leq \text{temperature} \leq 18^{\circ}\text{C}$ ($n=610$) were produced by taking averages of 20 adjacent points that were sorted by pollution or temperature.

London: 1965-1972, Spring/Fall ($4^{\circ}\text{C} \leq \text{temp.} \leq 9^{\circ}\text{C}$)

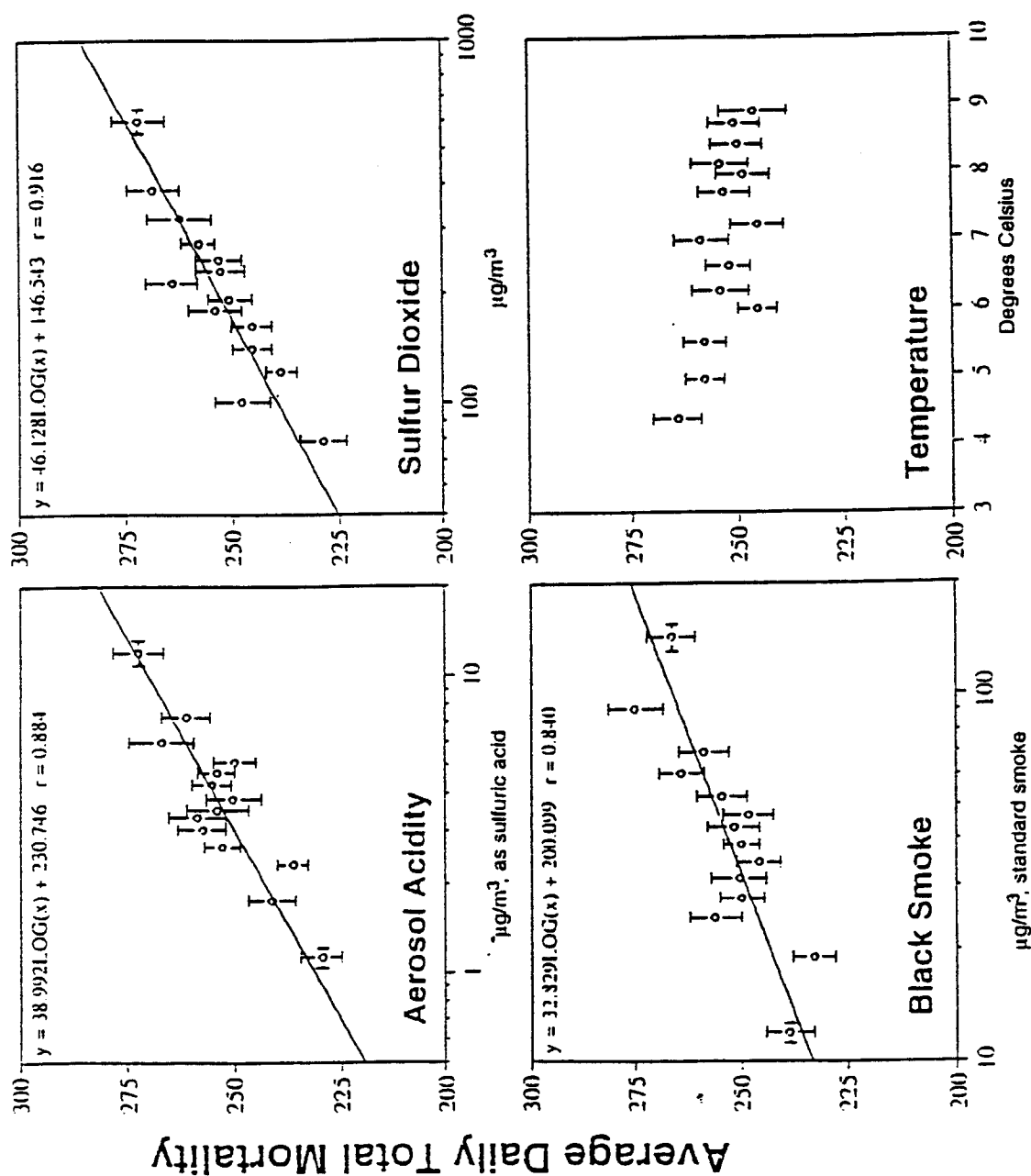


Figure 5. All Plots for spring/fall data where $4^{\circ}\text{C} \leq \text{temperature} \leq 9^{\circ}\text{C}$ ($n=275$) were produced by taking averages of 20 adjacent points that were sorted by pollution or temperature.

London: 1965-1972, Spring/Fall (12°C < temp.)

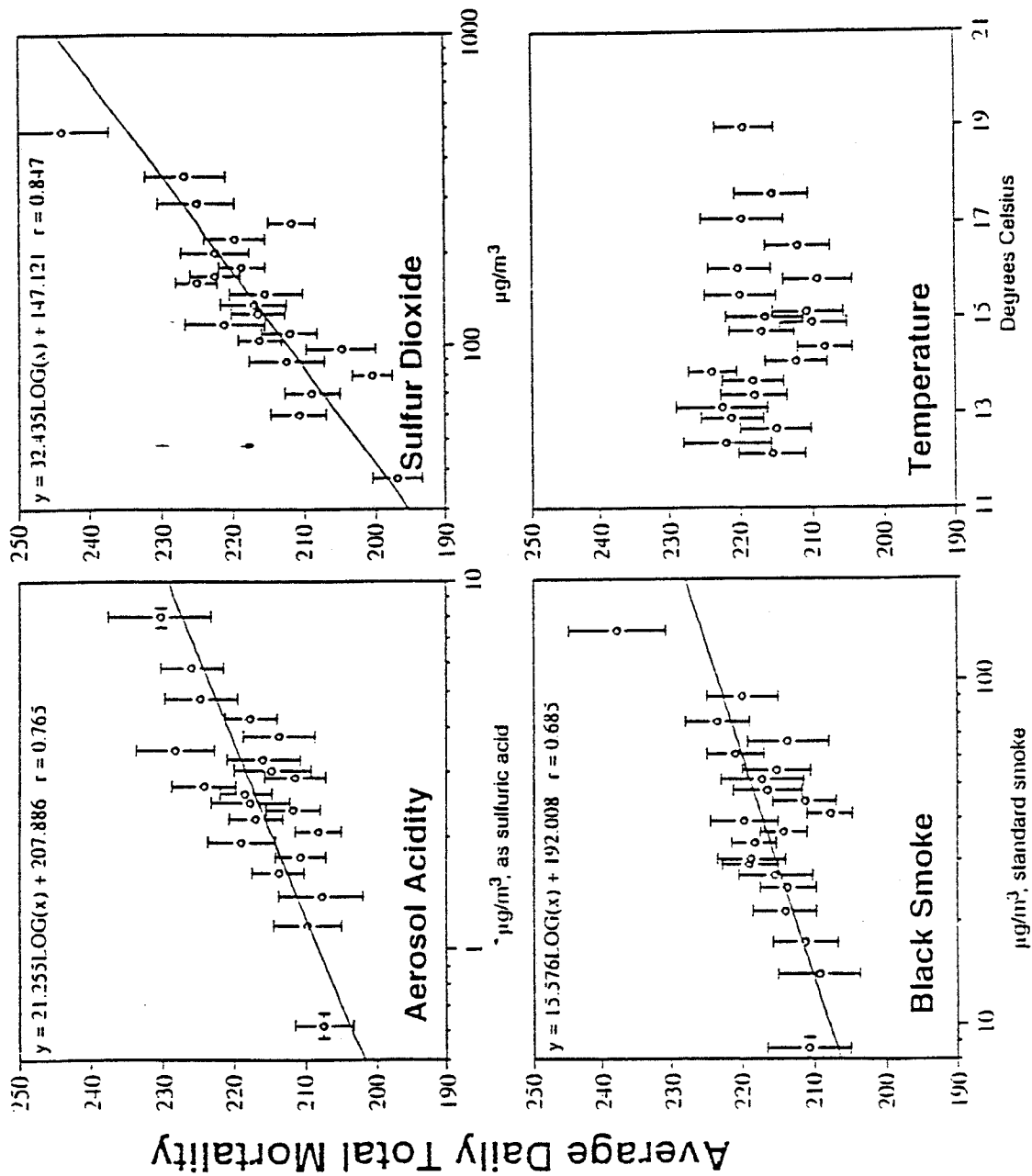


Figure 6. All Plots for spring/fall data where 12°C < temperature (n=118) were produced by taking averages of 20 adjacent points that were sorted by pollution or temperature.

TIME SERIES (1963 - 1991) OF MORTALITY AND AMBIENT AIR POLLUTION IN CALIFORNIA: AN ASSESSMENT WITH ANNUAL DATA.

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ABSTRACT

This paper discusses: i) a data base developed from individual mortality counts from 1963 to 1991, containing monthly and annual average mortality rates for several endpoints for several California counties, and monthly and annual air pollution and climatic data; and ii) descriptive statistical analyses conducted with annual average data. The major coastal urban and non-urban counties in California are included. In the north we include Alameda, Contra Costa, Marin, San Francisco, Santa Clara, San Mateo; and, in the south: Los Angeles, Orange, Riverside, San Bernardino, San Diego. The air pollutants include Total Suspended Particulate Matter (TSP), Pb, O₃ and Total Oxidants, SO₄, NO₃, and CO, measured at several stations within these counties. The climatic data include temperature and relative humidity. The mortality rates, defined by the International Classification of Diseases (ICD, Revisions 8 and 9) are standardized by sex, race, and age to the 1980 California population are those resulting from a number of selected "all causes", all cancers, lung and respiratory cancers, cardiovascular diseases, and chronic obstructive pulmonary diseases, by county of residence. The population at risk is abstracted from the US Bureau of the Census yearly age, sex, race-specific county level data and from intercensal estimates. The findings based on annual data indicate that, generally, the counties with less air pollution are characterized by higher mortality. These findings are counterintuitive. Therefore these results are currently being investigated on the basis of monthly average data to 1992, by extending the air pollution data base to include PM₁₀, Nitrates₁₀, and Sulfates₁₀, and by stratifying mortality by age groups.

INTRODUCTION

There is considerable ongoing concern and debate about the magnitude of the effect of air pollution on mortality. An aspect of the debate can be addressed by determining whether the directions of the long-term trends of mortality follow those of air pollution. If the air pollution trends are lower in areas where mortality is higher, then some causal or preventive factor other than (or in addition to) air pollution may be operating. Similarly, if an air pollution time series is declining while the mortality time series is increasing, then the causal association between air pollution and mortality is questionable. Our research attempts to clarify these long-term temporal relationships.

The literature most relevant to our work is summarized in Table 1. In general, these studies have used regression methods similar to those used in this paper. The findings reported in

this literature support the contention that exposure to particulate matter and other air pollutants such as O_3 , CO, and PM_{10} is associated with significant increases in total mortality (excluding non-natural causes), and mortality from cardiovascular diseases and chronic obstructive pulmonary diseases. Nevertheless, some of the results of these studies also report statistically insignificant results, an aspect corroborated by other studies, which suggest that at least some statistical associations are questionable on technical and biological grounds.

In this paper we discuss the development of a data base on long-term air pollution, climatic data, and mortality data from several California counties. The counties for which mortality data have been developed are, in the north: Alameda, Contra Costa, Marin, San Francisco, Santa Clara, San Mateo; and in the south: Los Angeles, Orange, Riverside, San Bernardino, San Diego. These counties are among the major coastal urban and non-urban counties in California included in this study. The mortality rates are those resulting from a subset of all causes, all cancers, lung and respiratory cancers, cardiovascular diseases, and chronic obstructive pulmonary diseases. The air pollutants used in the analyses based on annual data are: Total Suspended Particulate Matter, O_3 and Total Oxidants, SO_4 , NO_3 , CO, and Pb; temperature and relative humidity are also included.

The paper has two objectives: i) the description of the data base, and ii) and an analysis using annual data abstracted from that data base.

METHOD

In this section we describe the data base and the statistical methods utilized to develop the time series of annual data. The annual mortality rates used in this paper are those calculated for the period 1968 to 1991, and air pollution and climate data for the period 1963 to 1988. The full data base, namely monthly and annual averages, is now being extended to 1992.

Mortality Data and Rates (1968 to 1991)

The mortality data base consists of time series of average monthly and annual death rates, by county of residence at the time of death: namely, the expected number of deaths by category of disease (Table 2) per 100,000 individuals. Stratum-specific mortality rates from 1968 to 1991 were weighted by the 1980 age, race, sex California population to estimate standardized expected mortality rates for each county for each year and for each month. The

individual mortality counts were abstracted from the National Center for Health Statistics (NCHS, Record Layout Tables) public use tapes (UCLA Institute for Social Science Research, ISSR), and then summarized as annual averages.

The category mortality from "all causes" is included because several authors relate total mortality to air pollution, generally including all causes up to the International Classification of Diseases (ICD) 8000. We, however, exclude causes of deaths from: tuberculosis (ICD 0100 to 0199), other infectious and parasitic diseases (ICD 0000 to 0099, and 0200 to 1399), endocrine and nutritional diseases (ICD 2400 to 2699), mental disorders (ICD 2900 to 3199), pregnancy and childbirth complications (ICD 6300 to 6789), and musculoskeletal diseases (ICD 7100 to 7399). The rationale for these exclusions is that a review of the biomedical literature (Ricci, Catalano, and Kelsh, 1993) suggests that these mortality endpoints are unlikely to be causally associated with exposure to air pollution.

The expected annual and monthly death counts form the numerator of the rate. The denominator (the population at risk) is based on the decennial US. Bureau of the Census age-race-sex-county specific data (1970, 1980, and 1990). We developed the age-sex-race-county specific intercensal estimates using linear regression estimates derived from the census data. The population estimates for 1968 and 1969 were replaced by the 1970 data. The race adjustments are made for "blacks" and "whites;" mortality for Hispanics, Asians, and other groups are included with those of "whites." All rates are per 100,000 persons.

Air Pollution and Climatic Data (1963 to 1988)

The air pollution data base has been developed using the hourly or daily data made available by the California Air Resources Board. The air pollutants used in this paper are ozone or total oxidants, carbon monoxide, and total suspended particulate matter (TSP), sulfate, nitrate, and lead. Two data series were developed from data provided by the National Climatic Data Center (NCDC) for temperature and relative humidity. The relative humidity series consists of monthly averages for the hours of 04, 10, 16, and 22 Pacific Standard Time. The long-term records from the stations for stations in Marin, San Mateo and Contra Costa counties where either of lesser quality or short length of record and were therefore excluded.

The air pollution and climatic data were developed from long-term records at the following stations (with the county shown within the parentheses) were included in the data bases: Livermore (Alameda), San Jose (Santa Clara), San Francisco (San Francisco), Azusa (Los

Angeles), Los Angeles City (Los Angeles), Anaheim (Orange), Riverside/Rubidoux (Riverside), Fontana (San Bernardino), and San Diego (San Diego). Evaluating these data showed that they are not homogeneous in the sense that the instruments have been moved from one area to another, within the same town or city. For instance, San Francisco's air monitoring station was moved three times in the last 26 years. In other cases, the record is discontinuous: for example, Oakland did not report relative humidity data from 1981 to 1988. Moreover, air quality monitoring stations are not located contiguously to the climatic stations.

The concentrations of pollutants (ionic charge omitted) were measured at different time intervals. CO and O₃ and climatic data are reported hourly; TSP, SO₄, NO₃, and Pb data daily (every sixth day); and climatic data monthly. The air pollution and climatic data were assessed by developing their empirical distributions and summary statistical measures such as the mean and standard deviation (geometric for TSP, Pb, SO₄, NO₃, O₃, and CO; arithmetic for temperature and relative humidity). O₃ and CO hourly concentrations were then summarized as monthly means and as averages of the daily maxima and minima. Particulate concentrations, relative humidity, and temperature were summarized as monthly means, maxima, and minima.

The method for measuring ozone changed from coulometric potassium iodide (CU) to ultraviolet photometric (UV), at most stations in California, between 1975 and 1981. The California Air Resources Board converted all early data from total oxidants (which contains such extraneous substances as formaldehyde) to ozone, except for the sites in Los Angeles County. The statistical analysis of the raw data (including box-and whisker plots) do not show the change; in most cases, neither the median nor the shape of the distribution or the interquartile ranges detect the change in method. Also, the reporting limits are shown as zero concentration when the reading is below the minimum reporting limit, which is 1 ppm, 1 pphm, and 1 µg/m³, depending on the pollutant. The statistical issues that arise in these cases have been discussed by Catalano, Ricci, Sahl, and Kelsh (1992). The missing data leading to the results we discuss in this paper were resolved using splines and regression models.

In general, CO is less variable than other pollutants, over the periods of record and by station. Except for NO₃ and, in some cases, SO₄ there are relatively few observations outside 3 interquartile ranges; the variability for NO₃ and SO₄ is influenced by artifacts. The NO₃ data, particularly in the early periods of record, from 1971, were overestimated and

more recently, apparently underestimated. TSP monitoring is based on systematic 6th-day sampling, resulting in 61 observation per year; however, prior to 1977, many stations reported more than 61 observations per year.

Modeling with Yearly Data

We first assessed the data by developing the joint distribution of paired mortality rates (variables) through smoothing methods such as the Gaussian kernel estimator. This technique describes the shape of the empirical joint distribution of two variables as a bivariate joint distribution. For example, the surfaces developed for the combination of lung cancers and all cancers for Orange, Los Angeles, and San Francisco counties show that the joint distributions have two or three hills, instead of being symmetrical and bell-shaped. This information has been generated for all counties and various combinations of mortalities and has been useful in developing mathematical transformations of the data to reduce the number of peaks to one and to introduce symmetry about the mean of the joint distribution.

The second aspect of the analysis is the development of a model that can be used to describe and predict future values of mortality and air pollution. The method adopted is the univariate Auto Regressive and Moving Average (ARMA) model, which includes autoregressive (AR) and moving average (MA) processes. Taking disease-specific mortality rates, MR_t , (age-sex-race adjusted and standardized to the 1980 California population) as an example, a simple ARMA model is:

$$MR_t = c + b_1 MR_{t-1} + e_t - a_0 e_{t-1},$$

namely, ARMA of order (1,1). As shown, this model includes a one lag AR component (MR_{t-1}) and one MA disturbance (e_{t-1}); and the random error, e_t , is normally distributed. The essence of the analysis is to develop reliable and consistent estimates of the coefficients of this model (c , b_1 , and a_0) given data on MR and MR_{t-1} . The statistical estimator used to obtain the estimated values of the coefficients is the (non-linear) least squares; in this sense, the ARMA model is a "regression" model. This method is being extended, in the ongoing research, to establish and justify causal association via multivariate ARIMA models, using monthly measures of mortality and air pollution from the data bases discussed in this paper.

Before estimation, we developed autocorrelation and partial autocorrelation functions to determine the nature of the statistical processes underlying the time series, namely whether

AR, MA or both processes generated the time series. Second, we established whether the time series required differencing (and transformation) to achieve stationarity for the mean and the variance. First differences, taking the difference between two successive values of the dependent variable, generally were sufficient to yield stationary time series and thus we used the Integrated, Moving Average, Auto-Regressive (ARIMA). We also determined the statistically significant number of lags, periodicities (such as seasonal effects), and other technical characteristics of the ARIMA model, before estimation. The coefficients of each final ARIMA model (one for each mortality and for each air pollutant, per county) were estimated from the data up to 1988 through the non-linear least squares estimator (Box and Jenkins, 1976).

Using the ARIMA we predicted mortality values for three years (1989, 1990, and 1991). Those predicted values can both overestimate and underestimate the actual values depending on the relationship between the predicted value and the actual value for any of the three years. The over or under prediction is calculated by $[(\text{predicted value} - \text{actual value}) / (\text{actual value})]$, reported as a percentage.

We also developed simple and multiple regression equations in which specific mortality rates are regressed against a combination of pollutants to test the hypothesis that these pollutants are statistically associated with mortality. The air pollution variables are lagged and transformed by taking their natural logarithms. Because annual data consist of small samples, rather than assuming large sample (asymptotic) properties of the estimator, we used bootstrap methods (Efron and Tibshirani, 1993) to assess the consistency of the estimates we obtained by using small samples.

RESULTS

In this section we summarize the results from the annual time series of mortality and air pollution and the statistical analyses conducted with these data. Mortality from "all causes" is not reported because it is still under development; mortality rates for Marin County are excluded because we are not relating them to a local air pollution station or stations; San Diego, Contra Costa, and San Mateo counties, Los Angeles (Azusa) station air quality data, and the data sets for Pb and O₃ are not included in this paper for the sake of brevity.

Tables 3 and 4 depict some of the results obtained thus far using yearly data. The key finding is that the time series of average annual air pollution levels in the Northern California

counties, for the periods of record included in the data bases, are generally lower than those in Southern California. Yet, mortality rates for total and respiratory cancers are higher in San Francisco and Alameda counties than in any of the other counties included in this research, as are cardiovascular (CV) mortality rates. Our results are consistent with the magnitude of the rates and the finding that higher mortality rates occur in Northern California with the data provided by several statistical reports (*Cancer Incidence*, March 1993; *Cancer Mortality*, March 1993; Sotocky, 1989; Riggan, Van Bruggen, Acquarella, Baubier, and Mason 1983; and U.S. Dept. Health and Human Services, 1992). However, differences in standardization, reference population used, and disease codes included or excluded make the direct comparisons among the trends difficult.

Our results are counterintuitive: the working hypothesis is that high pollution levels would be associated with high disease-specific mortality rates. For this reason, we are continuing the quality controls and statistical analysis to discover latent structures in the data, possible errors and omissions.

Second, as an internal consistency check and using ARIMA models we predicted mortality rates from 1989 to 1991 and compared those predictions with the actual data for 1989, 1990, and 1991.

The results are gratifying. For example, recalling that a forecast can overpredict in one year and underpredict the next, total cancers mortality rates Los Angeles (LAC Station) are overpredicted between approximately 2% and 7%; Orange is overpredicted between 3% and 4%; Riverside is under and overpredicted between 1% and 3%; and San Bernardino follows a similar pattern, ranging between 1% and 3%. San Francisco total cancers mortality rates are generally overpredicted between 3% and 9%; Santa Clara is underpredicted between 4% and 11%; and Alameda is also underpredicted from 1% to 5%. Los Angeles mortality rates associated with respiratory cancers are over predicted between 2% and 7%; Orange's are overpredicted between 4% and 17%; Riverside's are underpredicted between 2% and 4%; while San Bernardino's overpredicted by 2% and underpredicted by 4%. These mortality rates for San Francisco are generally underpredicted between 10% and 20% and overpredicted by 22%; Alameda's are underpredicted between 2% and 5% and overpredicted by 2%; and those for Santa Clara are overpredicted between 5% and 13%. Roughly similar results are obtained for CV and COPD (Chronic Obstructive Pulmonary Diseases).

For respiratory cancer mortality and COPD mortality, the bootstrap results from the simple and multiple regressions confirm that small sample estimation results are consistent and quite similar, in terms of the direction of the relationship and the value of the coefficients. However, because TSP and SO_4 were collinear, we used simple regressions for COPD mortality and respiratory cancer mortality against lagged and log-transformed TSP and SO_4 . The results generally show that the individual coefficients were negative and statistically significant (at the 0.05 level of significance). These results were confirmed by the bootstrap estimates. When we regressed CV mortality against TSP, CO, SO_4 , and NO_3 , the associations with SO_4 and NO_3 were negative, statistically significant and generally unaffected by first order autocorrelations. However, the relationship between CV and NO_3 is affected by first order autocorrelation. The accuracy of these results was confirmed by bootstrap analyses. Additionally, the relationship between CV mortality and TSP or O_3 was positive and statistically significant; however, these results are affected by autocorrelation and by outliers. Further analyses are being conducted to remedy the effect of autocorrelation and to account for the outliers.

Third, we investigated the smoking and some socioeconomic temporal and spatial patterns. These were developed from data obtained from the National Health Information Service, from 1965 to 1987, from surveys conducted by the Oakland Kaiser Permanente Health Maintenance Organization, the Gallup organization, and from the literature. Overall, 29 studies were used to obtain data to be used in regression modeling (Ricci, Kelsh, and Bekken, 1991). Those regressions, for cities such as Alameda, San Francisco, Los Angeles, San Diego, and pooled areas, consisted of the number of packs of cigarettes smoked per day as a function of time, for male and female smokers. The results suggest that smoking trends were declining over time; although the sample sizes were small, the regression results were often statistically significant (Ricci, Kelsh, and Bekken, 1991). These data have been updating to 1993 are being studied in conjunction with the monthly air pollution and mortality trends and time series.

Currently, using monthly data, we are assessing age, race, and sex-specific trends over several age strata, and we are developing statistical analysis and modeling based on causal multivariate time-series. All the data bases are being updated to 1992 and the monthly air pollution data base has been extended to include the Coefficient of Haze (COH), light scattering, sulfates₁₀, nitrates₁₀, and PM₁₀. We are also studying the effect of small samples, interval censored and missing data, in the more recent data air pollution data, on the estimates of the parameters of the univariate and multivariate time-series through the Monte

Carlo and bootstrap methods (Little and Rubin, 1987) and other methods (Tsutakawa, 1988; Tsay, 1986 and 1987).

CONCLUSIONS

Because our research is in progress, the conclusion that follows is contingent on the continued analysis of the data base, quality controls, and statistical analysis of the time series based on monthly data. Nevertheless, the results that we report are counterintuitive: we expected high mortality rates to be associated with high pollution levels. This is generally not the case. Since our results consider exposures and effects over the long-term, and have been validated against similar time series reported by others, we feel that our overall results cast some doubt on the magnitude and the direction of the effect of ambient, low levels of exposure to TSP and the other air pollutants on the mortality endpoints we used. Nevertheless, since this research is continuing, our results are interim. We also recognize that the time series do not explicitly account for confounders and effect modifiers, but rather are indicators of the probable causal relationships between air pollution and mortality.

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Table 1, Summary of Selected and Recent Literature Relevant to this Research

Authors (Year)	Unit Of Analysis (City/County)	Mortality Rates (MR)	Included Air Pollutants	Main Findings
Shumway, Azari, and Pawitan (1988).	Los Angeles County data from 1970 to 1979.	Daily total, cardio-vascular (CV) and respiratory (resp.) mortality.	CO, SO ₂ , NO ₂ , hydrocarbons (HC), O ₃ , and particulate (as KM).	Positive correlations between total and CV MR and CO, HC, and KM.
Kinney and Özkaynak (1991).	Los Angeles County data from 1970 to 1979.	Daily CV, total and respiratory. (ICD 8 < 800; 390-459; and 460-519).	Total oxidants, SO ₂ , NO ₂ , CO, KM.	Significant association of MR and total oxidants, temperature, NO ₂ and relative humidity.
Schwartz (1991).	Detroit; ten-year period.	Daily total. (ICD 9 ≤ 800).	TSP, O ₃ , SO ₂ .	Previous day TSP and SO ₂ positively correlated with MR, but not with O ₃ .
Mills et al. (1991).	Los Angeles, six year period (7th day Adventists).	Malignant neoplasms, resp. cancers.	TSP, O ₃ .	"Borderline" statistical significant results for TSP.
Dockery, Schwartz, and Spengler, (1992).	St. Louis and eastern Tennessee counties, one year period.	Total daily (ICD ≤ 800).	PM ₁₀ , PM _{2.5} , SO ₂ , O ₃ , acid aerosols.	Seasonal temp. and PM ₁₀ were significant predictors of mortality.
Pope, Schwartz, and Ransom (1992).	Utah Valley, (1985 to 1989).	Total daily (ICD 9 ≤ 800), resp. diseases, and CV.	PM ₁₀ .	Significant results for resp. MR, CV MR, and Total MR.
Dockerey et al. (1993).	Six Cities Study, Data since 1974.	Total.	Sulfates, fine and inhalable particles.	Significant positive results.

Table 2. Mortality Endpoints Included in the Data Base and Used in this Research.

Mortality Endpoints	International Classification of Diseases (ICD) and Revisions to the Diseases Included ^a	ICD Codes Numbers Used to Develop the Annual Mortality Data Base
Respiratory Cancers	8	1600 - 1639.
	9	1600 - 1659.
Chronic Obstructive	8	4900 - 4939; 5180 and 5199.
Pulmonary	9	4900 - 4969.
Diseases (COPD)		
Cardiovascular	8	3900 - 4489.
Diseases (CV)	9	3900 - 4489.
All Cancers	8	1400-2399.
	9	1400 - 2399.
All Causes	8	See Text.
	9	

^a The ICD system classifies diseases by certain homogeneous groups. It is revised from time to time. The two classifications used reflect the inclusion or exclusion of certain diseases, within a specific classification, as the state of information changes. The ICD codes used are those that classified the original mortality data.

Table 3. Selected Summaries of Air Quality and Mortality Trends (Southern California)^a

County (Station)	Pollutants ^b	Years Included	1st Year- Last Year (Natural Trend) ^c	Mortality Rates	1st year - Last Year. (1968 - 1988) (Natural Trend)
Orange (Anaheim)	NO ₃	76 - 88	13.1 - 11.3 (-)	Total Cancers	142.8 - 160.6 (+)
	TSP	70 - 88	97.5 - 83.4 (-)	Resp. Cancers	32.8 - 44.3 (+)
	CO	63 - 88	5.1 - 1.6 (-)	COPD	23.5 - 29.5 (+)
	SO ₄	76 - 88	8.6 - 6.2 (-)	CV	450.9 - 257.9 (-)
Riverside	NO ₃	74 - 88	26.0 - 20.2 (-)	Total Cancers	151.1 - 161.5 (+)
	TSP	70 - 88	125.3 - 135.8 (+)	Resp. Cancers	31.7 - 48.6 (+)
	CO	63 - 88	3.0 - 1.4 (-)	COPD	29.3 - 35.3 9 (-,+) ^d
	SO ₄	74 - 88	9.8 - 6.4 (-)	CV	448.8 - 291.0 (-)
San Bernardino	NO ₃	75 - 88	10.1 - 18.8 (+)	Total Cancers	145.2 - 179.6 (+)
	TSP	72 - 88	140.2 - 135.1 (-)	Resp. Cancers	27.9 - 55.94 (+)
	CO	74 - 88	2.5 - 1.2 (-)	COPD	21.7 - 41.9 (+)
	SO ₄	75 - 88	10.1 - 6.1 (-)	CV	444.4 - 331.8 (-)
Los Angeles (LAC)	NO ₃	66 - 88	9.4 - 15.8 (+)	Total Cancers	166.5 - 165.8 (+)
	TSP	72 - 88	120.0 - 100.4 (-)	Resp. Cancers	32.7 - 44.0 (+,-)
	CO	63 - 88	2.2 - 2.1 (-)	COPD	19.8 - 27.1 (+)
	SO ₄	66 - 88	8.6 - 8.0 (-)	CV	519.6 - 303.6 (-)

^aThe data base includes data to 1991, see text for discussion.

^b Units in ppm (CO) or µg/m³; geometric annual averages or arithmetic annual averages; mortality rates are age, race, sex adjusted to the 1980 California population, per 100,000, by county of residence.

^c Natural trends of the untransformed data (the way the data actually look on visual inspection) characterized by +, - or vice versa show reversal of the trend about the early to mid 1970s or the early 1980s, depending on the station or county.

^d The general trend is positive, but the last years indicate a decline.

Table 4, Selected Summaries of Air Quality and Mortality Trends (Northern California)^a

County (Station)	Pollutants ^b	Years Included	1st Year- Last Year (Natural Trend) ^c	Mortality Rates	1st Year - Last Year (1968 - 1988)
San Francisco	NO ₃	71 - 88	1.3 - 3.0 (+) ^b	Total Cancers	204.5 - 190.3 (+,-) ^d
	TSP	71 - 88	48.1 - 42.6 (+,-) ^d	Resp. Cancers	43.1 - 51.8 (+)
	CO	64 - 88	4.5 - 2.0 (-)	COPD	25.4 - 27.5 (+)
	SO ₄	71 - 88	1.9 - 4.7 (+)	CV	536.6 - 483.7 (-)
Alameda (Livermore)	NO ₃	71 - 88	2.3 - 4.2 (+)	Total Cancers	183.6 - 182.5 (-)
	TSP	71 - 88	71.2 - 48.3 (-)	Resp. Cancers	38.1 - 51.4 (+)
	CO	71 - 88	3.5 - 1.1 (-)	COPD	21.0 - 34.5 (+)
	SO ₄	71 - 88	1.4 - 3.2 (+)	CV	535.6 - 284.5 (-)
Santa Clara (San Jose)	NO ₃	71 - 88	3.6 - 7.0 (+)	Total Cancers	167.0 - 151.5 (+,-)
	TSP	70 - 88	72.5 - 77.7 (+)	Resp. Cancers	31.1 - 39.6 (+)
	CO	64 - 88	3.4 - 1.6 (-)	COPD	16.6 - 27.1 (+)
	SO ₄	71 - 88	1.5 - 4.6 (+)	CV	439.8 - 261.3 (-)

^aThe data base includes data to 1991, see text for discussion.

^b Units in ppm (CO) or $\mu\text{g}/\text{m}^3$; geometric annual averages or arithmetic annual averages; mortality rates are age, race, sex adjusted to 1980 California population, per 100,000, by county of residence.

^c Natural trends of the untransformed data (the way the data actually look on visual inspection) characterized by +, - or vice versa show reversal of the trend about the early to mid 1970s or the early 1980s, depending on the station or county.

^d The general trend is positive, but the last years indicate a decline.

PARTICLES AND MORTALITY: A CLINICAL PERSPECTIVE

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ABSTRACT

Increased levels of particulate matter in the air have been associated with increased respiratory morbidity and mortality. The recent mortality findings are remarkable for the demonstration of an apparent adverse effect of particles in concentration ranges below the present National Ambient Air Quality Standard. This finding warrants consideration in the context of both our understanding of clinical disease and relevant data from toxicologic studies. The elderly and persons with severe chronic obstructive pulmonary disease (COPD) would be expected to be particularly at risk; causes of acute cardiopulmonary death might be attributed to pulmonary edema, acute respiratory infection, exacerbation of COPD or perhaps arrhythmias. Available toxicologic studies provide few clues in explaining acute mortality at low particle concentrations. Controlled clinical studies with acidic particles at concentrations greater than twenty times ambient fail to produce a pulmonary inflammatory response in healthy individuals; subjects with COPD, the group at presumably highest risk from the epidemiologic data, show no reduction of lung function with similar acute exposures. Perhaps our understanding of the toxicity of urban particles could be increased by investigations directed at the combined effect of particles with surface complexed metals, particles plus oxidants, sulfuric acid-coated ultrafine metallic particles, or even ultrafine particles alone. Despite the epidemiologic observations, from a clinical perspective the pathophysiologic basis for the excess cardiopulmonary deaths remains problematic; until the findings of new toxicologic studies become available, the framework for interpreting the epidemiologic findings will be inadequate.

INTRODUCTION

A series of recent reports have shown that variation of ambient PM₁₀ (particulate matter less than 10 µm aerodynamic diameter) even at current levels is positively associated with the variation of daily cardiopulmonary mortality and total mortality, excluding accidental and suicide deaths (Dockery and Pope, 1994). These associations have been shown in different communities including Philadelphia, St. Louis, the Utah Valley, and Santa Clara County, California, with widely differing particle composition and climates. Despite these epidemiologic findings for acute and chronic adverse health effects from air pollution associated with respirable particles, there are not complementary data from animal toxicologic and controlled human exposures to respirable particles. Controlled human exposures to acidic and inert particles have not caused significant alterations in respiratory function in healthy individuals or those with underlying chronic respiratory diseases. Furthermore, to date no mechanistic insights have emerged from either toxicologic or epidemiologic approaches to provide a biologic basis for interpreting the epidemiologic observations.

In this paper, we examine possible pathophysiologic mechanisms linking particulate air pollution and mortality, focusing on effects in susceptible populations. Secondly, we provide a brief review of controlled human exposure studies relevant to understanding the effects of inhaled particulate matter at current concentrations. We conclude by addressing the issue of biological plausibility and by considering future toxicologic research that might provide mechanistic insights into the unexplained observations.

PATHOPHYSIOLOGIC MECHANISMS

Reviews of the epidemiological studies linking air pollution and increased mortality have been published (Dockery and Pope, 1994). Most time-series have found the strongest relationship between particle concentrations and same-day mortality, although Pope et al. (1991) also observed a 2-3 day lagged effect on lung function. These findings imply that particle exposure may be "further compromising the health of persons who are already at significant risk of dying" (Schwartz and Dockery, 1992). As in the London Fog episode, the increases in death rates observed in these studies appear to be primarily attributed to cardiac and respiratory causes, particularly among the elderly. No data suggest that ambient air pollution is a direct cause of death in healthy people, even during the most severe historical episodes. Although particulate air pollution could conceivably contribute to the genesis of asthma, chronic bronchitis, or other airways diseases in susceptible individuals, this would require years of exposure, and would not explain the strong association with daily mortality. Smokers are exposed daily to concentrations of toxic particles many orders of magnitude greater than ambient; yet years of exposure are required before disease and increased mortality risk become evident, and only a minority of smokers appear to be susceptible.

The findings therefore suggest that certain segments of the population, particularly the elderly and those with underlying cardiac or respiratory disease, may be at risk of death from small increases in ambient particle concentrations. Because the strongest effect is seen on same-day mortality, effects must be manifested over a relatively short time period, i.e., less than 24 hours.

What mechanisms could be active in this phenomenon? Particle exposure could conceivably increase mortality in the following ways: 1) "premature" death for individuals already very near death (i.e., hastening of an already certain death by hours or days); 2) increased susceptibility to infectious diseases (i.e., influenza,

bacterial pneumonia, etc.); and 3) exacerbation of chronic underlying cardiac or respiratory diseases.

Death-bed effects

Particulate pollution could contribute to daily mortality rates by affecting those at greatest risk of death: individuals for whom death is already imminent. What may be only a minor irritant for healthy people could be the "last straw" that tips over the precariously balanced physiology of a dying patient. Such an effect would be analogous to the effect of temperature deviations. Time-series analyses have shown relationships between temperature deviations and increasing mortality regardless of the direction of temperature change, with magnitude similar to that described for particulate air pollution (Kunst et al., 1993). Although a few deaths can be attributed to hyperthermia or hypothermia, the excess mortality due to moderate temperature deviations is primarily among the chronically and terminally ill, likely caused by further stress on over-burdened physiologic compensatory mechanisms.

If particulate air pollution simply represents a physiologic stress similar to temperature deviations, and the excess mortality is occurring among individuals who would have died anyway within days or weeks, one would expect to see a "harvesting effect". In other words, following the increase in mortality associated with any given rise in particulate concentrations, the mortality rate should fall below baseline, because some of those at risk will have already died. In fact, although such an effect has been found with temperature-related mortality (Kunst et al., 1993), it has not been evident in the epidemiology of particulate exposure. Even in the 1952 London fog episode, there was no decline in mortality following the peak in excess deaths; instead, increased mortality appeared to remain somewhat elevated in the days after pollution levels had returned to baseline (Logan, 1953).

Other studies suggest the effect of particles on mortality cannot be solely explained by death-bed effects. In a longitudinal study, Dockery and colleagues found a strong association between particulate air pollution and mortality in 6 US cities, after adjusting for smoking and a number of other risk factors (Dockery et al., 1993). In addition, mortality and respiratory illness in the Utah Valley have been linked with particulate exposure associated with a steel mill (Pope, 1989). These findings indicate effects on annual mortality rates, and cannot be explained by effects on individuals already at death's door.

Increased susceptibility to infection

Particle exposure could increase susceptibility to infection with bacteria or respiratory viruses, leading to increased incidence of, and death from, pneumonia. However, pneumonia rarely results in death within 24 hours of onset; serious infections of the lower respiratory tract generally develop and evolve over days and weeks, and would not explain effects on daily mortality. If pollutant exposure increased susceptibility to specific infectious diseases, it should be relatively easy to detect differences in the incidence of such diseases in communities with low vs. high particulate concentrations, given appropriate controls for other contributing factors. Emergency room visits and hospitalizations for pneumonia caused by the relevant agent should be measurably higher on high particle days. No such relationships have been observed. Furthermore, animal exposure data to support such a mechanism are lacking. Although exposure to acidic aerosols has been linked with alterations in mucociliary clearance (Spektor et al., 1989), non-acidic aerosols and other particulate species have not been shown experimentally to result in increased susceptibility to infection.

Enhanced severity of underlying respiratory disease

Finally, particulate air pollution could aggravate or enhance the severity of underlying respiratory disease. This mechanism could explain increases in daily mortality (through effects on those near death from their disease), and longitudinal increases in mortality (if individuals with chronic airways disease experienced more frequent or severe exacerbations of their disease, or more rapid loss of function as a result of particulate exposure).

What chronic disease processes are most likely to be affected by inhaled particulates? In order to explain the daily mortality statistics, these must be common conditions that contribute significantly to overall mortality from respiratory causes. The most likely candidates are the chronic airways diseases, particularly chronic obstructive pulmonary disease (COPD). COPD is the fourth leading cause of death in the U.S., and is the most common cause of non-malignant respiratory deaths, accounting for more than 84,000 deaths in 1989 (U.S. Bureau of the Census, 1992). This group of diseases encompasses both emphysema and chronic bronchitis, and information available on death certificates generally does not allow differentiation between these diagnoses. The pathophysiology includes chronic inflammation of the distal airways as well as destruction of lung parenchyma. There is loss of supportive elastic tissue, so that airways collapse more easily during expiration, obstructing outflow of air. Processes that enhance airway inflammation or edema, increase smooth muscle contraction in the conducting airways, or slow mucociliary clearance could adversely affect gas exchange and host defense. Moreover, the uneven ventilation - perfusion matching characteristic of this disease, with dependence on fewer functioning airways and alveoli for gas exchange, means inhaled particles may be directed to the few functioning lung units in higher concentrations than in normal lungs (Bates, 1992).

Smoking is the most important etiology of this group of diseases, and many patients continue to smoke until very late in their disease. It is difficult to conceive that a small increase in ambient particles could measurably affect the health of individuals whose lungs are being exposed to the very high concentrations of particles associated with cigarette smoke. Even if the physiology associated with well-established COPD were to make the lung uniquely susceptible to the adverse effects of particle exposure, one would expect that smoking a single cigarette, with inhalation of particle concentrations many times higher than ambient, would be lethal. This does not appear to be the case.

Asthma is a common chronic obstructive respiratory disease that may be exacerbated by air pollution. Mortality from asthma has been rising in recent years (Gergen and Weiss, 1992), and air pollution has been implicated as a potential causative factor. Atmospheric particle levels have been linked with increased hospital admissions for asthma, worsening of symptoms, decrements in lung function, and increased medication use (Bates et al., 1987; Pope et al., 1991; Schwartz et al., 1993). However, asthma is often a disease of the young and otherwise healthy. Mortality, although increasing, is uncommon, accounting for approximately 3% of total respiratory deaths in 1989 (U.S. Bureau of the Census, 1992). It is therefore unlikely that the observed mortality increases can be accounted for by increased deaths due to asthma alone.

Cardiovascular mortality

Increases in cardiovascular mortality have been described in association with particulate pollutants, both in the major air pollution episodes and in the more recent time-series analyses. Bates (1992) has postulated three ways in which pollutants could affect cardiovascular mortality statistics: 1) acute airways disease misdiagnosed as pulmonary edema; 2) increased lung permeability, leading to

pulmonary edema in people with underlying heart disease and increased left atrial pressure; and 3) acute bronchiolitis or pneumonia induced by air pollutants precipitating congestive heart failure in those with pre-existing heart disease. In addition, the pathophysiology of many lung diseases is closely intertwined with cardiac function. For example, one postulated cause of the increasing mortality rate in asthma is overuse of adrenergic agonist medications leading to fatal cardiac arrhythmias.

Many individuals with COPD also have cardiovascular disease caused by one or more of the following: 1) smoking, 2) aging, or 3) pulmonary hypertension accompanying COPD. Terminal events in patients with end-stage COPD are often cardiac, and may therefore be misclassified as cardiovascular deaths. Hypoxemia associated with abnormal gas exchange can precipitate cardiac arrhythmias and sudden death.

Another example of a respiratory disease with important cardiac complications is obstructive sleep apnea. This condition is under-recognized and very common, with prevalence estimates ranging between 1% in Israeli industrial workers and 42% in an elderly nursing home population (Redline et al., 1993). It is caused by narrowing of the upper airway often related to fat deposition, particularly in very obese individuals. The relaxation of upper airway musculature during the rapid-eye-movement phase of sleep leads to upper airway obstruction, apnea, hypoxemia, and subsequent arousal. Chronic sleep deprivation accounts for most associated symptoms. However, some patients with this disorder are at significant risk of death from cardiac arrhythmias precipitated by the nocturnal hypoxemia. It is conceivable that relatively minor insults to the airways, including increases in particulate air pollution, could either worsen upper airway obstructive episodes, or worsen the nocturnal hypoxemia, leading to a fatal cardiac arrhythmia. Because the underlying respiratory condition often goes unrecognized, many deaths from this disorder may

be misclassified as cardiac deaths. However, any possible effects of inhaled particles in this disease remain conjectural.

In summary, epidemiological studies have provided compelling evidence of a link between ambient particulate air pollution and mortality, but causality has not been established. If the relationship is causal, those most likely to be affected are individuals with common, chronic respiratory diseases, the course and severity of which could be worsened by exposure to particles. The observed increases in cardiovascular mortality are likely to represent misclassification of cause of death, and/or effects on individuals with respiratory disease complicated by cardiac disease.

CONTROLLED HUMAN EXPOSURES TO PARTICLES

Carefully-controlled, quantitative studies of exposed humans provides an informative approach to evaluate responses in individuals with acute and chronic respiratory disease (Utell et al., 1993). This experimental approach has the potential to test the hypothesis that individuals with chronic lung disease are susceptible to low-level particle exposure. Human clinical studies utilize laboratory atmospheric conditions, which are considered relevant to ambient pollutant atmospheres, and document health-relevant effects that result from breathing the atmospheres. Advantage is taken of the highly-controlled environment to identify responses to individual pollutants and characterize exposure-response relations.

Diverse aerosols are produced by air pollution, gas phase reactions and dispersion of organic dust, e.g., tree resins, pollens, decaying vegetations and spores. Of this mixture of natural and anthropogenic aerosols, clinical studies have tended to examine primarily the latter category, especially inorganic sulfates and nitrates, which play a prominent role in "acid rain". Asthmatic subjects have been found most susceptible to the effects of acidic aerosol exposure, although different laboratories have found differing threshold exposure concentrations. In general,

however, studies of adult asthmatics have failed to demonstrate alterations in lung function at levels below $200 \mu\text{g}/\text{m}^3$. Utell and co-workers (1983) exposed asthmatics to H_2SO_4 , NH_4HSO_4 , NaHSO_4 , and a control NaCl aerosol at concentrations of 100, 450 and $1,000 \mu\text{g}/\text{m}^3$. Following exposures to the 450 and $1,000 \mu\text{g}/\text{m}^3$ aerosols for 16 minutes, specific airway conductance decreased in relation to the acidity of the aerosol, supporting the hypothesis that airway effects are related to acidity rather than the sulfate ion. More prolonged exposures to H_2SO_4 aerosols have also been performed (Morrow et al., In press). With 10-minute exercise periods every 30 minutes, a 2-hour exposure to $100 \mu\text{g}/\text{m}^3$ H_2SO_4 aerosol resulted in a small reduction in flow rates. The lung function response to H_2SO_4 was maximum after the first 45 minutes, followed by a return towards baseline. Thus the effect did not appear to be progressive over time. Respiratory ammonia was identified as a factor influencing responses to sulfuric acid aerosols; by reducing the level of endogenous respiratory ammonia, airway responses to inhalation of $350 \mu\text{g}/\text{m}^3$ aerosols in exercising asthmatics were enhanced (Utell et al., 1989). This provided further evidence that bronchoconstriction was related to acidity of the inhaled sulfate aerosol.

Adolescent asthmatics appear to be more sensitive to the effects of acidic aerosols than adult asthmatics. Functional decrements have been observed in adolescent asthmatics following exposure to H_2SO_4 aerosols at levels as low as $68 \mu\text{g}/\text{m}^3$ for 40 minutes (Koenig et al., 1989). The apparent difference in sensitivity of adult and adolescent asthmatics may be due to differences in subject selection, aerosol sizes or exposure protocols, but adolescent asthmatics respond to concentrations of H_2SO_4 aerosols at an order of magnitude lower than normal subjects. However, in these studies young asthmatics demonstrated functional decrements at exposure levels near peak outdoor levels in the Northeastern U.S. Field studies in summer camps of both normal and asthmatic children reported decrements in pulmonary function during pollution episodes that included increased

levels of acidic aerosols (Raizenne et al., 1989), reinforcing concern that children and adolescents may be particularly susceptible to effects of acidic atmospheres.

Collectively, these data demonstrate that asthmatics manifest significant, albeit small, reductions in their lung function during moderate physical activity after inhaling acidic aerosol concentrations close to high ambient levels. For asthmatics, the irritant potency of sulfate aerosols appears related to acidity per se. However, the decrements in lung function are relatively small after exposure to acid particles, are not progressive, and are mitigated by ammonia neutralization. These findings therefore are not consistent with the epidemiologic findings of increased mortality and morbidity related to ambient particle exposure.

Based on the epidemiologic observations, subjects with COPD are considered to be potentially at high risk for mortality and morbidity from particles. To determine whether low-level H_2SO_4 aerosol inhalation induced alterations in lung function in volunteers with COPD, older subjects (mean age = 62 years) were exposed to $90 \mu\text{g}/\text{m}^3$ H_2SO_4 aerosols in an environmental chamber for 2 hours (Morrow et al., In press). Subjects were defined by dyspnea on exertion, obstructive airways disease ($\text{FEV}_1 = 1.4$ liters or 53% predicted and $\text{FEV}_1/\text{FVC} = 0.56$) and a lack of response to bronchodilators. In contrast to findings in asthmatic patients, the volunteers with COPD demonstrated no greater decrements in pulmonary mechanics in response to H_2SO_4 than to the control aerosol, sodium chloride. Subjects with COPD, presumably the most vulnerable subpopulation, demonstrated virtually no change in flow rates even with periods of intermittent mild exercise during their exposure periods. Therefore, in the only study evaluating responses to specific components of particulate matter, enhanced responsiveness could not be identified.

DISCUSSION AND CONCLUSION

In considering the biological plausibility of this new evidence on adverse effects of particles, we emphasize the following principles: consistency with clinical observations, consistency with other observed effects of air pollutants on humans, and consistency with toxicological investigations using animal models. The most perplexing observation in regard to these criteria is that of excess daily mortality associated with 24-hour average particulate concentrations as low as $50 \mu\text{g}/\text{m}^3$. Although there is no question as to the plausibility of a causal relationship between higher levels of particulate exposure and excess mortality, for example, in the London smog episodes with particulate concentrations as high as $4000 \mu\text{g}/\text{m}^3$, the causal relationship extending to concentrations as low as $50 \mu\text{g}/\text{m}^3$ is problematic. Indeed, individuals at risk for mortality from particles would be expected to be indoors and the contribution of ambient particles to personal exposures would be further reduced, recognizing that 100% penetration from the outside is highly unlikely even for fine particles.

Particle dosimetry in the respiratory tract is an excellent starting point for discussions of biological plausibility, as it addresses key issues from a perspective independent of specific particle composition. When different ventilatory regions of the lung are compromised with respect to their ventilatory capacity, such as in pre-existing asthma, COPD, or congestive heart failure, those regions of the lung that are still healthy can receive a disproportionately high dose of particles placing those remaining healthy regions at even greater risk and thus, further compromising the lungs reserve capacity. However, an increase of ambient particle concentrations of $20\text{-}40 \mu\text{g}/\text{m}^3$ would translate into relatively small increases in peripheral lung particle deposition; from a pathophysiologic perspective, it seems unlikely that such particle concentrations could worsen ventilation-perfusion ratios in healthy or injured lung to the point of producing hypoxemia sufficient to cause pulmonary edema due to left

ventricular failure, increased permeability, or malignant arrhythmia. Similar clinical arguments exist when attempting to relate exacerbations of respiratory infections to increased particle loads.

Alterations in host defense mechanisms is another important factor in biological plausibility. Only one study has been performed to examine effects of H₂SO₄ aerosols on host defense mechanisms at the alveolar level (Frampton et al., 1992). Non-smoking volunteers were exposed for 2 hours to an aerosol of approximately 1,000 µg/m³ H₂SO₄ or NaCl, with intermittent exercise, in a random double-blind fashion. Bronchoalveolar lavage (BAL) was performed 18 hours after exposure in order to detect evidence of an inflammatory response, change in alveolar cell subpopulations, or changes in alveolar macrophage function, all components of host defense. The investigators concluded that brief exposures to H₂SO₄ aerosols at 1,000 µg/m³ did not cause an influx of inflammatory cells into the alveolar space, and no evidence was found for alteration in alveolar antimicrobial defense 18 hours after exposure. In a similar study, Sandstrom and Rudell (1991) examined the bronchoalveolar inflammatory response to inhalation of diesel exhaust. Diesel exhaust from an idling diesel engine was diluted with air and introduced into an exposure chamber. Median concentrations in the breathing zone were 3 x 10⁶ particles/cm³, 3.7 ppm NO, 1.6 ppm NO₂, 27 ppm CO, and 0.3 mg/m³ formaldehyde. Exposures were performed for one hour and included moderate exercise on a bicycle for 10 minute periods alternating with rest. Lavage was performed 18 hours after exposure and demonstrated an increase in neutrophils in the bronchoalveolar but not the bronchial portion of the lavage. The abstract indicates that phagocytosis of opsonized yeast cells in vitro by macrophages from the bronchoalveolar lavage was significantly altered. Although exposures to pollutant gases such as SO₂ or NO₂ alone have typically not induced an acute inflammatory response, the interactions of particles, gases and hydrocarbons in causing responses to diesel exhaust is unclear.

Therefore, neither clinical experience nor review of the literature identify a supportable pathophysiologic mechanism to explain the relationship between inhaled particles and mortality. There are, however, limitations in the toxicologic data base. Toxicologic studies directly relevant to ambient particles are sparse and fail to replicate ambient particle mixtures. None of the animal and human studies have used particle generation systems that reflect the complexity of ambient particles. Nevertheless, it seems unlikely that there is a highly toxic and still unidentified particle that fails to produce even an inflammatory response in normal airways but when deposited in injured airways can cause death.

A few recent animal toxicologic studies provide new insights for future research. Studies with real-world particles with surface complexed iron (Tepper et al., 1994), ultrafine Teflon particles (Oberdoerster et al. In press), or sulfuric acid-coated ultrafine metallic particles (Amdur and Chen, 1989) provide interesting models linking a specific component of real-world particulate matter with frank inflammatory effects in animals. Novel approaches provide opportunities to identify mechanisms of injury including characterization of the role of mediators, the identification of neurotransmitters, and an understanding of the subtleties of immune suppression. Such techniques could eventually uncover mechanisms by which particles alone or complexed with metals could provoke pulmonary edema, arrhythmia or exacerbation of severe obstructive airways disease. For the present, however, such mechanisms remain highly speculative, and largely theoretical. Until findings of new toxicologic studies become available, the framework for interpreting the epidemiologic findings will be inadequate (Utell and Samet, 1993).

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TOXICOLOGICAL EVIDENCE FOR HEALTH EFFECTS FROM INHALED
PARTICULATE POLLUTION: DOES IT SUPPORT THE HUMAN
EXPERIENCE?

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Abbr. Title: Toxicology of Ambient Particles

ABSTRACT

The fine size mode of ambient particulates, designated as PM₁₀, is a heterogeneous mixture that can vary in particle size and chemical composition, depending upon geographical location, meteorology, and source emissions. While epidemiological studies indicate an association between ambient particulate air pollution exposure and increased human mortality and morbidity, the question remains as to biological plausibility. Individual components of PM₁₀ have been shown, in experimental studies, to produce what may be considered to be adverse health effects similar to those observed in humans. For example, diesel exhaust particles are associated with lung tumors, and acid sulfates are associated with airway hyperresponsiveness and alterations in mucociliary clearance. However, in most cases, exposure levels are well above those found in ambient air, and the relationship between results of toxicological assessments and human exposure scenarios is not always clear. Furthermore, in some cases, the chemical species resulting in adverse responses in toxicological studies are not always the same as those associated with effects in human population studies. Thus, while toxicological evidence clearly indicates the ability of certain ambient particles to induce increased morbidity in laboratory studies, the responsible chemical species have not been definitively delineated.

INTRODUCTION

Over the past few years, a number of epidemiological studies have concluded that ambient particulate exposure is associated with increased mortality and morbidity (e.g., Pope III et al., 1991; Dockery et al, 1993; Ostro, 1993; Schwartz, 1993). These studies have been conducted in several different geographical locations and have involved a range of populations. While the consistency of the findings and the presence of an apparent dose-response relationship provides a strong argument for causality, epidemiological studies can only conclude this based upon inference from statistical associations.

About 30 years ago, in a presentation before the Royal Society of Medicine, Sir Austin Hill, a professor of medical statistics at the University of London, remarked, "It would be helpful if the causation [between the environment and disease] we suspect is biologically plausible, but biological plausibility depends upon knowledge of the day." (Hill, 1965). Thus, any cause-effect relationship discerned from epidemiology would be greatly strengthened by the existence of plausible, underlying biological mechanisms for particulate-associated adverse health effects. Obtaining these becomes the purview of toxicology, which can serve to provide mechanistic links between exposure to ambient particulate pollution and increased human morbidity and mortality. The question at hand, however, is whether the available toxicological evidence "of the day" is consistent with the epidemiology.

There is toxicological evidence for adverse health effects from polluted air. For example, rats exposed for six months to the ambient atmosphere of São Paulo, Brazil showed bronchial secretory cell hyperplasia, increased mucous secretion and decreased mucociliary function; these changes were not observed in rats exposed to cleaner rural air (Saldiva et al., 1992). Mice exposed to Los Angeles air showed increased number and area of Type 2

pulmonary cells, which may be an index of early lung injury, compared to animals exposed to the cleaner air of Santa Barbara (Sherwin et al, 1991). These two studies are, in a sense, the toxicological equivalent of epidemiology, and while they may indicate that daily exposure to "real" ambient air can result in pulmonary effects, the role of particles in producing these cannot be determined. On the other hand, numerous controlled toxicological investigations of individual chemical species have clearly shown that specific constituents of ambient particulate matter are associated with adverse biological effects.

Since fine particles may pose a greater health risk than coarser ones, the national ambient air quality standard is measured in terms of particulate matter having an aerodynamic diameter equal to or less than 10 μm , an aerometric termed PM_{10} . But the PM_{10} mode of ambient air is, in reality, a heterogeneous mixture that varies in constituent particle sizes and chemical composition, depending upon geographical location, meteorology and source emissions. It can include trace metals, e.g. copper, iron, nickel, and vanadium; carbon particles from motor vehicle engine exhaust; organics from incomplete combustion and photochemical reaction processes; nitrates; and sulfates. Part of the problem in obtaining plausible mechanisms for the human experience has to do with this heterogeneous nature of particulate matter in the atmospheres examined, and the inability of the available epidemiological studies to definitively relate effects to any individual chemical species, or combination of species, within this mixture.

To address the issue of biological plausibility it is first necessary to understand which specific human health effects are associated with particulate exposure. Mortality correlated with particulates is due largely to cardiovascular/pulmonary disease and lung cancer (Dockery et al, 1993; Ostro,

1993), although the latter may compromise the lungs making them vulnerable to other effects of particles. Nonlethal health endpoints include decreased pulmonary function; increased use of asthma medication and increased incidence of asthmatic attacks; increased hospital admissions; increased chronic cough, congestion and phlegm production; acute bronchitis in children; increased risk of chronic bronchitis; and increased risk of cancer (Schwartz, 1993).

The goal of this paper is to provide a broad framework for attempting to determine where the relevant toxicological database stands relative to the epidemiological, i.e., whether the available toxicological studies are able to provide some explanation for the biological effects noted above. It is not intended to be a comprehensive review of inhaled particle toxicity, a topic which could occupy numerous volumes. Rather, the approach is to indicate whether specific constituents of the fine particulate mode have, in experimental studies, been shown to result in biological responses which may be the same as, or analogous to, those seen in humans exposed under ambient conditions.

PARTICULATE EXPOSURE AND RELEVANT BIOLOGICAL RESPONSES

Is there evidence for carcinogenicity of ambient particles?

Perhaps the one component of the particulate mode which is most associated with carcinogenicity is diesel engine exhaust (Klingenberg and Winneke, 1990; Scheepers and Bos, 1992; Mauderly, 1992). This is actually a complex mixture, consisting of carbon particles, onto which are adsorbed various organic chemicals, plus a number of associated gases. In order to determine the role of particles *per se* in producing adverse health effects, some studies have examined diesel exhaust from which the gases were

removed. Exposure to diesel exhaust particles (DEP) are associated with the development of lung tumors in some rodents following long-term exposures at high concentrations, i.e., above $\sim 1 - 2 \text{ mg/m}^3$.

Two mechanisms may underly DEP carcinogenicity (Mauderly, 1992). A genotoxic mechanism suggests that tumor development results from the interaction of particle-associated organics with pulmonary cell DNA, while an epigenetic mechanism suggests that tumors follow the overloading and retardation of lung clearance, subsequent accumulation of particles, inflammation, and the interaction of inflammatory mediators with cell proliferative processes and DNA. These two mechanisms may not, however, be mutually exclusive, in that carcinogenesis could be the net effect of the parallel action of direct genotoxic damage and indirect damage mediated by the immunoresponse of the lung to the inhalation of large numbers of fine particles.

A dual mechanism for DEP-induced cancer in rodents is supported by the observation that when tumor rates are assessed as a function of the total dose of DEP delivered, i.e., exposure concentration times exposure duration, a threshold is noted below which no carcinogenic effect is found, a phenomenon which contradicts the accepted notion for carcinogens acting via a direct genotoxic mechanism. On the other hand, such a threshold could be explained by a retardation in normal lung clearance mechanisms when exposure concentration reaches a certain level, resulting in abnormal particle accumulation. If this latter is actually the case, then the responses to DEP at high concentrations cannot be extrapolated to ambient levels, since with realistic exposures, the lung's normal defenses would remain operational and particles would not accumulate to the same extent. To put exposure concentrations into some perspective, an average DEP concentration at street

level near a heavily traveled road could be $15 \mu\text{g}/\text{m}^3$, and the cumulative exposure for a person remaining in this location for 3 hr/d for 70 yr would be about $1.1 (\text{g} \times \text{hr})/\text{m}^3$; this is well below the threshold exposure level of $8 (\text{g} \times \text{hr})/\text{m}^3$ required for the initial incidence of lung tumors in rodents (Klingenberg and Winneke, 1990). Thus, there is little evidence for the potential carcinogenicity to humans of DEP under realistic ambient exposure conditions.

Inhalation of other types of particles, even those having low intrinsic toxicity, has been shown to produce pulmonary inflammation when ultrafine sizes (i.e., $<0.02 \mu\text{m}$) are employed, but exposure concentrations were generally in the mg/m^3 range and exposure durations were quite long, on the order of months (Ferin et al, 1992; Oberdörster, 1988). Recently, however, acute (0.5 hr) inhalation of freshly generated ultrafine polytetrafluoroethylene particles at $4\text{--}8 \mu\text{g}/\text{m}^3$ resulted in pulmonary inflammation and mortality in rats (Oberdörster et al, 1994). But the relationship between results with such particles and ambient exposures to more inert materials is not clear.

Is there evidence for effects of ambient particles on pulmonary function?

Most toxicological studies of particles have not examined effects on pulmonary function. However, two major components of PM_{10} in many regions are nitrates and sulfates, and pulmonary functional responses to these have been assessed.

The limited data for nitrates indicates that acute exposures to very high concentrations, up to about $4,000 \mu\text{g}/\text{m}^3$, do not alter pulmonary function in dogs or mice (Sackner et al, 1976; Ehrlich, 1979). Similarly, sulfate aerosols have generally not been associated with alterations in pulmonary function indices following exposures below $1,000 \mu\text{g}/\text{m}^3$. The notable exceptions to the

latter are studies of Amdur and colleagues, in which single exposures to fine sulfuric acid particles at concentrations down to $100 \mu\text{g}/\text{m}^3$ often resulted in increased airway resistance in guinea pigs (Amdur, 1989). Furthermore, Amdur and Chen (1989) showed that repeated, daily 3 hr exposures of guinea pigs to a lower concentration, namely $20 \mu\text{g}/\text{m}^3$, of an ultrafine ($<0.1 \mu\text{m}$) aerosol of sulfuric acid coated on metal particles resulted in reduced vital capacity. In another study, sulfuric acid was suggested to be the responsible chemical species in the reduction of both vital capacity and carbon monoxide diffusion capacity noted in guinea pigs exposed for 2 hr to ultrafine coal fly ash (Chen et al, 1990).

What may be concluded from the toxicological studies of sulfates and nitrates is that, most likely, exposure concentrations needed to alter pulmonary function in healthy animals are well above ambient levels [which are $50 - 75 \mu\text{g}/\text{m}^3$ peaks for sulfuric acid (Spengler et al, 1989), and $10 - 35 \mu\text{g}/\text{m}^3$ for nitrates (Schlesinger, 1992a)]. The exception is the guinea pig, which is often considered to be a model for the sensitive human and, in this regard, may indicate potential effects on certain subpopulations.

Is there evidence for the induction or exacerbation of chronic obstructive lung disease by ambient particles?

One component of PM_{10} for which there is a fairly robust toxicological database suggesting a potential role in the pathogenesis of chronic obstructive lung disease is the acid sulfates. Exposure to sulfuric acid in particular has been associated with a number of biological alterations analogous to those seen in humans having asthma or chronic bronchitis.

Fine sulfuric acid particles have been demonstrated to induce a state of nonspecific airway hyperresponsiveness, a hallmark of human asthma, in

otherwise healthy animals. Rabbits exposed to $250 \mu\text{g}/\text{m}^3$ for 1 hr/d for 4 months showed increased responsiveness in one study (Gearhart and Schlesinger, 1989), while in another, single 3 hr exposures to $75 \mu\text{g}/\text{m}^3$ also resulted in evidence for hyperresponsive bronchi (El-Fawal and Schlesinger, 1994). The underlying mechanism appears to be interference with normal contractile/dilatory homeostatic processes in the airways via modulation of airway receptors involved in maintenance of airway tone. The development of hyperresponsive airways in healthy nonatopic animals at an acid sulfate exposure level below that producing any change in other aspects of lung function clearly has implications for the pathogenesis of airway disease due to nonspecific irritant exposure.

One of the suggested potential mechanisms underlying pollutant-induced exacerbation of asthmatic symptoms may be increased airway epithelial permeability, with subsequent enhanced penetration of inhaled antigens, present in most ambient atmospheres, to submucosal cells involved in allergic reactions (Balmes, 1993). Certain fine particles have been associated with permeability alterations. For example, rats exposed to $70 \mu\text{g}/\text{m}^3$ ammonium sulfate ($0.2 \mu\text{m}$) or to $350 \mu\text{g}/\text{m}^3$ ammonium nitrate ($0.6 \mu\text{m}$) for 4 hr/d, 4d/wk for 8 wk showed evidence for such increased permeability (Kleinman et al, 1994).

Chronic bronchitis is characterized by dysfunction of tracheobronchial mucociliary clearance, and both short and long-term exposures to sulfuric acid have been shown to alter the rate of mucociliary transport. The response is related to both exposure concentration and exposure time, with low exposures accelerating clearance and higher ones retarding it. The lowest concentration for sulfuric acid found to alter mucociliary clearance is $200 \mu\text{g}/\text{m}^3$ for a single 1 hr exposure, and $100 \mu\text{g}/\text{m}^3$ for 1 hr daily

exposures (Schlesinger, 1989). Furthermore, the slowing of clearance seen in repeated exposure studies appears to persist beyond the period of acid sulfate exposure (Gearhart and Schlesinger, 1989). While the pathobiological significance of transient alterations in mucus transport in healthy individuals is uncertain, persistent impairment of clearance may lead to the inception or progression of respiratory disease and, as such, is a plausible link between inhaled acidic sulfates and chronic obstructive lung disease.

In the repeated exposure studies, alterations in mucociliary clearance are accompanied by histological changes in the bronchial tree, characterized by persistent hypertrophy and hyperplasia of epithelial secretory cells. (Gearhart and Schlesinger, 1989). This is associated with a shift in the histochemistry of mucus from neutral to acidic; the latter is more viscous than the former, and such change in rheology may underlie the altered mucus transport rate. Whether these cellular changes are permanent is currently not known, but persistently increased secretory cell number in peripheral airways and excess mucus production, a likely consequence of this increase, are sequelae of chronic bronchitis in humans.

It should be mentioned that pure sulfuric acid, which is often used as the model for acid sulfates in toxicological studies, rarely exists in ambient air. The acidic sulfate component usually consists of ammonium bisulfate or a mixture of neutralized sulfates, which are less potent than sulfuric acid (USEPA, 1989). Nevertheless, acidic sulfate particles can induce changes in the lungs of healthy animals which are consistent with human asthma and chronic bronchitis. Furthermore, the effects appear to be cumulative during each exposure day, at least in part, and the total exposure conditions in toxicological studies are within, or not far above, the range of actual human ambient exposure experience. For example, daily 1 hr exposures to $250 \mu\text{g}/\text{m}^3$

in rabbits may very well be equivalent to $<50 \mu\text{g}/\text{m}^3$ for a seven to eight hour day, and to a still lower concentration for equivalent effects in humans (Lippmann et al, 1987).

Is There Evidence for Immunosuppression by Ambient Particles?

Some of the morbidity/mortality seen in epidemiological studies may reflect dysfunction in host resistance, resulting in increased susceptibility to cancer or infectious disease. While, as discussed above, acidic sulfate particles have been shown to alter mucociliary transport, an essential defense function of the respiratory tract which to some extent is involved in antibacterial defense, other aspects of lung defenses have been shown to be affected by components of the ambient particulate mode.

Many trace metals are immunosuppressive, with a number of studies showing that metal particles, or carbon-coated with metals, are cytotoxic to macrophages and can increase susceptibility to respiratory infections (Goyer, 1986; Schlesinger, 1990). However, the short term inhalation exposure levels needed to produce any response generally ranged upward from $100 \mu\text{g}/\text{m}^3$, well above ambient concentrations (Goyer, 1986). While long-term exposures to some metals in occupational settings have indicated carcinogenic potential, exposure concentrations were much higher than those experienced by the general population (Nemery, 1990).

Exposure to nitrates and acidic sulfates has been associated with altered macrophage functions important for maintaining host resistance against infectious agents, although in most cases exposure levels were much higher than ambient. Pulmonary macrophages obtained from rabbits exposed to sulfuric acid aerosol at $75 \mu\text{g}/\text{m}^3$ for 2 hr/d for 3 days showed reduced amounts of reactive oxygen intermediates and had reduced activity of tumor

necrosis factor (Zelikoff et al, 1992). Additionally, macrophages from rabbits exposed to sulfuric acid aerosol at 750 or 1,000 $\mu\text{g}/\text{m}^3$ for 4 d (2 hr/d). showed reduced uptake and intracellular killing of bacteria and reduced biological activity of interleukin-1, a cytokine involved in cellular defense (Zelikoff et al, 1994). Nitrate particles may also modulate reactive oxygen products derived from pulmonary macrophages (Kleinman et al, 1994).

Modulation of pulmonary pharmacological receptors could be involved in particulate-induced effects related to altered lung defenses. For example, β -adrenergic stimulation down-regulates pulmonary macrophage function, and this has been shown to be influenced by short term, repeated exposures to sulfuric acid aerosol (McGovern et al., 1993). The acid-induced enhanced down-regulation may create an environment conducive to secondary pulmonary insult, such as bacterial infections, especially in susceptible populations. In addition, alterations in mediator release from the macrophage due to receptor down regulation may, in turn, influence airway responsiveness.

SOME ISSUES IN THE DESIGN AND INTERPRETATION OF TOXICOLOGICAL STUDIES IN SUPPORT OF EPIDEMIOLOGY

The advantages of toxicological studies in assessing biological responses to particles are obvious. They allow for exposures to strictly controlled atmospheres, a high flexibility in choosing exposure levels, the possibility of performing long term exposures that may result in persistent biological changes, the ability to examine a wide range of endpoints, and the clear linkage of response to specific agents. However, results of such studies cannot be directly applied to humans, and must be extrapolated. Furthermore, certain

choices made in study design may influence the results, and it is not always clear how these choices relate to actual human exposure scenarios.

Although most of the toxicological data concerning particles have been obtained from exposures using single compounds, it is important to understand responses resulting from inhalation of pollutant mixes to which humans are more likely exposed. But the toxicology of chemical mixtures is fairly complex, in that the occurrence and extent of interaction between mixture constituents is highly dependent upon the endpoint being examined and may also depend upon the concentration of the individual pollutants within the mixture (Schlesinger, et al, 1992b). It is likely not possible *a priori* to predict the nature of any interaction merely based upon stated exposure conditions.

While studies with binary mixtures are the most common, more realistic exposures are to multicomponent mixtures; but all possible combinations of toxicants cannot be experimentally examined. Furthermore, results of studies using such combinations are often difficult to interpret due to chemical interactions between the components and the difficulty in controlling or defining the ultimate composition of the exposure atmosphere.

The results of toxicological studies with particles are clearly influenced by choices made concerning specific exposure parameters, such as particle size. For example, the toxicity to rats of freshly generated ultrafine polytetrafluoroethylene particles was reduced when the aerosols were allowed to age, resulting in an increase in particle size (Oberdörster, et al, 1994). A synergistic increase in the severity of pulmonary lesions in rat lungs exposed to both sulfuric acid and ozone were noted when the acid particle size was 0.06 μm compared to 0.3 μm (Kimmel et al, 1994). While a particle size dependence of toxicity may relate to deposition differences in the lungs, it

may also reflect differences in the number of particles in the exposure atmospheres, in that both number concentration and mass concentration may affect the response to inhaled aerosols (Chen et al, 1994). Exposure duration is also a factor in modulating response, with some effects noted in acute studies that seem to adapt with repeated exposures, or persistent changes seen with long-term studies that do not occur with shorter term exposures. Thus, the nature of the "educated guess" in any study design can result in apparent consistency, or lack of it, between toxicological studies and epidemiology.

CONCLUSION: DOES TOXICOLOGY PROVIDE BIOLOGICAL PLAUSIBILITY IN SUPPORT OF EPIDEMIOLOGY?

The question posed at the beginning of this paper is whether current toxicological evidence exists to show biological plausibility in support of epidemiology or, to put it another way, whether the epidemiological cause and effect relationships are consistent with available toxicological knowledge concerning the health effects of particulate air pollutants. As mentioned, this paper is not all inclusive, and does not consider all possible components of the fine particulate mode of ambient air nor does it address dosimetric differences between humans and animals used in toxicological studies.

With this in mind, the answer to the above question is both *yes* and *no*. The *yes* is that there is clear evidence that specific components of the fine ambient particulate mode, or PM₁₀, alone, or as components of mixtures with other pollutants, have been shown to produce adverse biological responses consistent with human morbidity findings. These are, for example, associations between genotoxicity with diesel exhaust particles; airway hyperresponsiveness, altered mucociliary transport and secretory cell hyperplasia with acid sulfates; and immunosuppression with acid sulfates

and metals. The *no* is that, in most cases, the exposure concentrations or total exposures needed to produce any effect were well above those experienced by the general human population and that, in some cases, normal homeostatic defense processes were affected, making it difficult or impossible to relate responses at these levels to those occurring at more realistic exposure concentrations. But a *caveat* is the acid sulfates, where consistent biological responses were noted at exposure conditions close to those to which the general human population may experience.

In terms of plausibility for increased human mortality, there are no toxicological data to allow any conclusion. However, most toxicology studies examined healthy adult animals, rather than animal models of susceptible human populations, such as the elderly or those with preexisting respiratory or cardiac disease, groups in which particulate-associated mortality is generally manifest (Schwartz, 1994). There is also a lack of long-term studies with many potential toxicants at low concentrations, or perhaps lack of study of appropriate mixtures.

It is clear that more targeted studies are needed to get a better handle on the specific components of PM₁₀ which may be responsible for observed human effects in certain populations. But there is the beginning of biological plausibility in support of epidemiology, with links between certain pollutants, e.g. particulate associated acidity, at somewhat realistic exposure levels and biological responses consistent with epidemiological findings. As a famous scientist in another discipline, Sherlock Holmes, once remarked, "When you have eliminated the impossible whatever remains, however improbable, must be the truth". While toxicological studies have not as yet indicated the impossibility of specific components of PM₁₀ in producing increased mortality/morbidity, they have indicated that some, such as diesel exhaust

particles, are probably not likely to be responsible, while others, such as acidic sulfates, are more consistent with human data. This is where the issue stands today.

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CELLULAR AND IMMUNOLOGIC INJURY WITH PM10 INHALATION

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Abstract

Airborne particles less than 10 μm (PM10) in mass median aerodynamic diameter (MMAD), are associated with adverse effects on human health. These include chronic lung diseases and mortality, but the mechanisms by which these particles cause or exacerbate diseases are not known. PM10 represent a complex mixture of particles, both in terms of size and chemical composition, and contains both aqueous-media soluble and insoluble particles. Furthermore, the ambient aerosol composition varies markedly in different locations and at different times in the same location. To test the effects of PM10 on pulmonary defenses in relation to specific cell targets, barrier-reared Sprague-Dawley rats were exposed to purified air (control), and to two important constituents of the fine particle ($< 1 \mu\text{m}$ MMAD) fraction of PM10 - ammonium sulfate $[(\text{NH}_4)_2\text{SO}_4^{-2}]$ (20 or 70 $\mu\text{g SO}_4^{-2} \text{ m}^{-3}$, 0.2 μm MMAD) and ammonium nitrate $[\text{NH}_4\text{NO}_3^{-1}]$ (90 or 350 $\text{NO}_3^{-1} \mu\text{g m}^{-3}$, 0.6 μm MMAD). Rats were also exposed to resuspended road dust (300 and 900 $\mu\text{g m}^{-3}$, 4.0 μm MMAD), an important contributor to the coarse ($> 2.5 \mu\text{m}$ MMAD) fraction of PM10. Exposures were 4 hr per day, 4 days per week for 8 weeks. Macrophage-dependent lung defense functions (antigen binding to Fc receptors and respiratory burst activity) were significantly depressed by SO_4^{-2} , and the high concentration SO_4^{-2} and road dust exposures, respectively, compared to purified air controls. Lung permeability, as determined from measurements of albumin concentrations in bronchoalveolar lavage fluid, was significantly greater in rats exposed to high concentrations of road dust and NO_3^{-1} , but not to SO_4^{-2} , when compared to air-exposed controls. Quantitative histopathologic analyses, which included measurements of alveolar nuclear density, alveolar chord length, alveolar septal thickness and alveolar cross sectional area, showed moderate to substantial changes. In general, the severity of the responses was in the order of $\text{SO}_4^{-2} > \text{NO}_3^{-1} > \text{road dust}$. This study supports the hypothesis that the fine fraction of PM10 is more toxic than the coarse fraction, and that exposures to PM10 components can affect respiratory defenses.

Introduction:

In southern California, the air quality standard for airborne particles less than 10 μm in diameter (PM₁₀) is one of the most frequently violated of the State's ambient air standards. Inhalation of PM₁₀ at levels near the State's (50 $\mu\text{g}/\text{m}^3$ 24 hr average) and often below the Federal government's (150 $\mu\text{g}/\text{m}^3$, 24 hr average) ambient air quality standards are associated with increased hospital admissions and emergency room visits for respiratory illnesses (Martin 1964; Greenberg et al. 1967; Samet et al., 1981; Knight et al. 1989), increased incidences of asthma attacks (Whittemore and Korn, 1980; Schenker, 1993), increased asthma medication use (Pope et al., 1991), reduced pulmonary function (Pope et al., 1991; Stern et al., 1989; Pope and Kanner, 1993), increased daily mortality (Schwartz, 1991; Schwartz and Dockery, 1992; Schwartz, 1993; Dockery et al., 1993). Exposure to respirable fine particles and sulfates are consistently and significantly associated with reported total annual mortality rates (Ozkaynak and Spengler, 1985; Ozkaynak and Thurston, 1987). People whose deaths have been associated most significantly with PM₁₀ exposures have been generally over 60 years of age, often bedridden (at home or in a medical facility) and often with pre-existing cardiovascular or chronic pulmonary disease (Utell, 1994).

The State, as well as the federal, standard is expressed in terms of sampled mass, and do not discriminate between particles of different chemical composition or different sizes within the overall particle diameter (d_p) \leq 10 μm size classification. The health effects associated with PM₁₀ toxicity are likely to be dependent upon both chemical composition and particle size. However, laboratory studies have not demonstrated convincing mechanistic links between exposure to PM₁₀ components and pathology. The association between PM₁₀ exposure and adverse health effects has not been previously tested in a systematic manner to determine whether the effects were attributable to specific components of the ambient aerosol and whether specific aspects of the lung's defensive systems were impaired. Such studies could elucidate the mechanisms by which PM₁₀ might be associated with acute mortality. This study, therefore, used the rat as a model to assess the potential of selected PM₁₀ components for modifying biological responses which could be related to the mechanisms by which

PM10 might affect human health. The primary hypothesis tested was that PM10 toxicity would be dependent upon the chemical composition of the aerosol.

The relationship between PM10 inhalation and acute mortality is an area of extreme concern. The mechanisms by which inhaled particles can precipitate fatalities are not clear, however there are some likely possibilities. For example, inhaled particles might impair the integrity of the lung's epithelial barrier causing increased leakage of serum and proteins into the lung ultimately leading to pulmonary edema and death. Impairment of pulmonary host defenses by inhaled particles could permit development of acute respiratory infections, or could cause acute inflammatory reactions with the release of reactive oxygen species and cytotoxic chemicals into the lung. These could lead to the exacerbation of existing chronic pulmonary diseases. It is also possible that mediators and cytokines released by particle-injured pulmonary cells could enter the circulation and cause cardiac dysrhythmia. These acute effects are typically seen in humans with a long lasting exposure to ambient particles. We therefore decided that an extended subacute study might be a better model for PM10 exposure than a single acute exposure. We therefore planned studies of an 8 week duration.

This study analyzed the effects of three PM10 components (ammonium sulfate, ammonium nitrate and road dust) which were selected because they represented important fractions of ambient PM10 aerosols. Two concentrations of each component were used. Rats were exposed at a low level which was representative of estimated peak 4 hr concentrations, based on extrapolations from ambient air data, and at a high level which was based on estimated worst case peak concentrations. The sizes of the particles used were chosen based upon reported sizes of fine and coarse mode inorganic aerosols in ambient air (John et al., 1990). The primary endpoints which were measured included: assessing changes in airway permeability; histopathological alterations in the lung, including thickness and cellularity of alveolar walls, alveolar chord lengths and alveolar cross-sectional diameters; and assays related to host defenses (macrophage Fc receptor binding capacity and release of biocidal compounds such as superoxide anion during respiratory bursts). Exploratory endpoints which were measured, but which did not show any significant PM10-related responses, included determinations of the numbers of

goblet cells in the respiratory epithelium, releases of the inflammatory mediators PGE₂ and LTB₄ and measurements of respiratory frequency and tidal volume during exposure.

Methods

Atmosphere generation and characterization: Ammonium nitrate and ammonium sulfate aerosols were generated by nebulization of dilute aqueous solutions. Road dust, which was collected by vacuum sweeping of freeway surfaces by CALTRANS¹, was disaggregated, sieved, and sterilized. The freeway sampled was relatively new and dust was collected from a region that had not been subject to any spills of hazardous materials. Road dust aerosol was produced using a custom built fluidized bed generator. Particles thus generated were passed through a vertical elutriator at a linear rate of flow equivalent to the settling velocity in air of 10 µm diameter unit density particles. The nitrate, sulfate and road dust particles were mixed with dry dilution air, brought to Boltzmann charge equilibrium by passage through a ⁸⁵Kr aerosol charge neutralizer, equilibrated to 60% relative humidity and then introduced into the exposure system airstream.

Size-classified aerosol samples were collected biweekly using a cascade impactor (Sierra Model 226 and Andersen Model 298; Graseby/Andersen, Atlanta, GA). A light-scattering aerosol monitor (RAM-1; MIE Inc., Billerica, MA) provided real-time mass concentration data during each exposure. Integrated 4 h samples of aerosol particles were collected daily from the rat's breathing zone on preweighed and equilibrated (50% R.H.) Pallflex T60A20 Teflon-coated glass fiber filters (Pallflex Co., Putnam CT). The filters were equilibrated to 50% R.H. and weighed to determine total mass. The filters were extracted with dilute aqueous 3 mM Na₂HCO₃, 2 mM Na₃CO₃, and the extracts were analyzed for SO₄²⁻, and NO₃⁻ by ion chromatography. Elemental carbon was determined on samples collected on quartz-fiber filters. These filters were heated to 850°C in pure oxygen and the resulting CO₂ quantified using a modified infra-red absorption monitor (Model 3003, Dasibi Environmental, Glendale CA). Samples of road-dust collected on membrane filters from the breathing zones of rats

¹ The State of California transportation authority.

during exposures were analyzed for elemental components by X-ray fluorescence spectrometry. Ozone was monitored using a UV absorption monitor (Model 1003AH, Dasibi Environmental, Glendale CA).

Animal Housing and Exposure: A total of 176 male, 200 to 250 g, specific pathogen-free F344/N rats (Simonsen Laboratories, Inc., Gilroy, CA), barrier reared and maintained in laminar flow isolation units supplied with filtered air, were randomly assigned to treatment groups (n = 10 to 12 per group) and exposed nose-only². Groups were exposed to high or low concentrations of pollutants. Exposures were 4 hours per day on 4 consecutive days per week for a total exposure time of 32 days over an 8 week period. Rats were exposed to purified air, road dust, nitrate or sulfate. Separate groups were assessed for histopathology endpoints and for macrophage and permeability-related endpoints. The high concentration groups were tested in one set of exposures, and the low concentration groups were tested in a separate set of exposures. Separate purified air control groups were used for high and low concentration exposures. Between exposures, rats were housed in a purified air-barrier environment and had access to clean water and dry laboratory chow *ad lib*. The vivarium was a AAALAC accredited and all animal protocols were reviewed and approved by an Institutional Review Board.

Necropsy, Tissue Preparation, and Morphometric Analysis:

Rats were killed 1 to 2 h after the end of exposure. Rats were anesthetized with IP pentobarbital (75 mg/kg body mass), and the tracheas were tied off just below the larynx. The lung and trachea were removed and fixed by inflation with neutral buffered formalin (pH 7.2) at a constant 30 cm H₂O pressure for 72 hr. After fixation, the left lung was sectioned longitudinally along the lobar bronchus and embedded in paraffin. Sections (5 µm thick) were cut from the blocks (1 section per block per animal) and stained with alcian blue/periodic acid Schiff (ABPAS) reagent. Section area was measured from slabs before embedding and from slides after staining, and shrinkage during embedding did not differ among groups. Lung sections were analyzed morphometrically using stratified random

² Nose-only exposures were used to prevent artifacts due to airborne dander, ammonia and dried excreta in the inspired air.

selection of 25 fields per section and avoiding bronchi and large blood vessels. Measurements included alveolar wall thickness, alveolar wall cellularity (nuclear density), alveolar chord length and alveolar cross sectional area as described by Rasmussen and McClure (1992).

Macrophage Function Analysis:

Bronchoalveolar lavage was performed on rats ($n = 12$ per atmosphere) to obtain macrophages for immunological testing and proteins for assessment of epithelial permeability³ (Kleinman et al., 1993). The rats were anesthetized, the abdominal aortas were severed, and the tracheas were exposed. A catheter was inserted into the trachea and tied in place. The lungs were lavaged with HEPES-buffered (pH 7.2) Hank's Balanced Salt Solution (HBSS) without Ca^{2+} or Mg^{2+} (GIBCO). The lavage volume was 7 ml and it was instilled and aspirated three times at a rate of about 0.5 ml/second. The lavage was repeated three times per animal and the recovered fluid from each lavage was held on ice. The lavage fluid from each animal was centrifuged at $300 \times g$, 4°C , for 10 minutes. The fluid from the first lavage was reserved for protein and biochemical assays. The cell pellets from all three lavages were pooled and resuspended in 3 mL HBSS with Ca^{2+} and Mg^{2+} (GIBCO).

The cells were counted using a bright line hemocytometer. Viability was assessed by Trypan Blue exclusion. The cell suspension was adjusted to 1 million viable cells per mL. The yield by this lavage procedure was typically 3 million cells per rat, of which more than 95% were macrophages with an average viability of greater than 90%. A 0.1 mL aliquot of cells was plated onto a glass microscope slide using a cytocentrifuge (Shandon Southern). The cells were stained with Wright-Giemsa stain and a differential count was made using previously described procedures (Nadziejko et al., 1992; Kleinman et al., 1993).

Changes in functional characteristics of alveolar macrophages (12 rats per atmosphere) were quantified by a rosette assay (Prasad et al., 1988, Kleinman et al. 1993) for determining Fc receptor binding capacity, and by determination of the production of superoxide anion during respiratory burst

³ Rats used in the lavage procedures were separate from those used in the morphometric measurements.

activity. To measure the ability of macrophages to bind sheep red blood cells (SRBC) to Fc receptors, adherent macrophages (10^5 cells) were incubated in microtiter chambers (Nunc, Inc.) with rat anti-sheep red blood cell antibody (30 min. @ 37°C) to allow the antibody to bind to the macrophage Fc receptors. The excess, unbound, antibody was removed by rinsing, and the macrophages were incubated with 10^6 SRBC's (30 min. @ 37°C). Excess red blood cells were removed by rinsing, and the number of macrophages forming rosettes with 3 or more SRBC were counted out of a total sample of 300 cells per chamber.

Superoxide anion production was measured using a cytochrome C reduction method (Nadziejko et al., 1992; Kleinman et al., 1993), as follows. Aliquots (100 μl) of the cell suspension (10^6 macrophages per ml) from each exposed rat were added to the wells of a 96 well microtiter plate. These wells then received 50 μl of cytochrome C (200 μM in HBSS) and 50 μl of freshly opsonized zymosan (5 mg/ml), and the cultures were incubated in the dark at 37°C for 60 min. Absorbance at 540 nm and 550 nm were read using a microtiter plate reader. The difference in absorbance ($A_{550} - A_{540}$) was multiplied by the molar extinction coefficient for reduced cytochrome C and the results were expressed as n moles/mg protein/60 minutes.

Permeability Methods:

Permeability was determined by measurement of albumin concentrations in the BAL using an enzyme-linked immunosorbent assay (Bhalla et al., 1992). Polystyrene nonflexible 96 well microtiter plates (Costar) were coated with 100 μl of 2 ng/ μl goat anti-rat antibody to albumin (Cappel) in carbonate buffer, pH 9.6. The plates were covered and refrigerated overnight (18 hours). The plates were washed two times with freshly made carbonate buffer. Non-specific binding was blocked by adding 150 μl of a gelatin-carbonate buffer (4 mg gelatin per ml carbonate buffer) to each well, which was then removed by washing two times with a solution of PBS-Tween 20-gelatin (0.5 ml TWEEN-20/L PBS + 1.0 mg Gelatin per ml) after 2 h incubation at 20°C . Serial dilutions of a standard (15 mg/ml) rat albumin (Sigma Chemical Co.) from 1:128,000 to 1:4096,000 and rat lavage fluid from 1:800 to 1:3200 were made using PBS-Tween 20-gelatin solution. Each well received 100 μl of the diluted standard or lavage fluid. The plates were covered and incubated in a humid chamber for 1 to 2

hours at room temperature and then washed three times with PBS-Tween-20-gelatin. A 1:2000 dilution of rabbit anti-rat IgG-peroxidase conjugated albumin (5.0 ml/ml) was made and 100 µl was added to each well. The plates were incubated in a humid chamber at room temperature for 1 hour, washed 2 times with PBS-Tween-20-gelatin and once with PBS-Tween-20. The color was developed by the addition of 100 µl per well of citrate-phosphate buffer (pH 5.0) which contained 1 mg/ml O-Phenylenediamine dihydrochloride (OPD) and 1 µl/2 ml of 30% H₂O₂. The plates were covered and incubated in the dark at room temperature for 20 minutes. The reaction was stopped by the addition of 50 µl per well of 2N H₂SO₄. The plates were read at 492 nm with a Titertek Multiscan MC plate reader.

Statistics:

Data were analyzed using analysis of variance with atmosphere as the independent variable. Direct comparisons between high and low concentrations were not made since the exposures were performed separately. The Dunnett multiple comparison test was used for *a-posteriori* testing of differences between atmosphere group means and their respective control group means. The criterion for statistical significance was set at $p \leq 0.05$.

Results

PM₁₀ is a complex mixture of primary emission particles such as combustion aerosols, particles generated by motor vehicles (tire wear, brake linings, elemental carbon from exhaust fumes), fugitive dusts resuspended from paved and unpaved roads by automotive activity or resuspended soil from agricultural or other sources, and secondary aerosols such as nitrates sulfates, and carbonaceous particles from atmospheric gas to particle conversion processes. While nitrates and sulfates dominate the submicron particle mode of PM₁₀, crustal type materials comprise the bulk of the supermicron size particles. In urban areas the largest source of the crustal components is resuspended dust from paved roads. The chemical composition of road dust can be quite variable, but in general resembles crustal material contaminated with organic and inorganic residues from motor vehicles and comminuted

paving material. The concentrations and aerodynamic diameters of PM10 and the nitrate, sulfate and crustal components measured during intensive sampling campaigns in three heavily polluted southern California communities are shown in Table 1. The sizes and concentrations of nitrate, sulfate and road dust (used as a surrogate for crustal material), to which rats were exposed, are also shown in Table 1.

Histopathology

Detailed morphometric analyses were performed on lungs of rats exposed to purified air or to the high concentrations of PM10 components. The results demonstrate significant ($p < 0.05$) PM10-induced changes. Alveolar wall nuclear density (Figure 1a) was increased slightly, but not significantly by road dust and sulfate, but was significantly increased by nitrate. Alveolar septal wall thickness was increased significantly by all three PM10 components (Figure 1a). Although the nitrate-exposed rats tended to exhibit greater increases in both nuclear density and wall thickness than the sulfate or road dust-exposed groups, the group mean values were not significantly different. Both alveolar chord length, a measure of average alveolar linear dimension, and alveolar cross sectional area tended to decrease in the groups exposed to PM10 components as compared to groups exposed to purified air; groups exposed to sulfate showed a significant decrease in area and groups exposed to nitrate showed significant decreases in both chord length and area (Figure 1b). Goblet cells were measured but no exposure-related differences were observed (data not shown).

Macrophage Function

Cells were recovered from rats by bronchoalveolar lavage. On the average, viability was greater than 90%, there were no exposure-related increases in numbers or percentages of lymphocytes or polymorphonuclear cells (PMNs), and macrophages represented between 95% and 100% of the recovered cells. About 3×10^6 macrophages were recovered in the lavage. Macrophages from rats exposed to PM10 components exhibited depressed ability to attack antigenic material (SRBC) via Fc receptor-mediated processes (Figure 2). A significant ($p = 0.018$) main effect of atmosphere was

observed after the low concentration exposures, but paradoxically, effects after high concentration exposures were not statistically significant. The group mean effects were small, overall, and at the low concentration, significant reductions were seen only after exposures to SO_4^{2-} . The production of superoxide during respiratory burst activity (Figure 3) was significantly depressed by high concentrations of SO_4^{2-} and road dust ($p < 0.05$). Exposures to all three pollutants at low concentrations tended to increase production of superoxide by macrophages, but the changes were not significantly different from the purified air group values.

Permeability

Bronchoalveolar lavage fluid (BAL) was analyzed for albumin, which served as an indicator of epithelial disruption and increased mucosal permeability. The results are shown in Figure 4. Increased ($p < 0.05$) permeability was observed in rats exposed to high concentrations of road dust and nitrate-containing atmospheres. After low concentration exposures, albumin level in the lavage fluid from all of the exposure groups were slightly, but not significantly, elevated when compared to the controls.

Discussion

This study demonstrated several biological effects in rats exposed to selected components of PM₁₀ and provided insights into the mechanisms linking ambient PM₁₀ exposures with increased acute human morbidity and mortality. The histopathologic changes observed in this study included thickening of alveolar walls and decreases in alveolar lumen cross sectional area. These findings could result from a decrease in lung compliance or "stiffening" of the lung which, under constant fixative inflation pressure, could become fixed with a smaller inflation volume. The possible loss of compliance could be due to early fibrogenic changes in the lung, or it could be due to increased infiltration of fluid and cellular components associated with the increased permeability we observed. The latter mechanism is consistent with our observation of increased alveolar wall nuclear density. It is likely that both factors may contribute, since permeability changes may be early indications of inflammatory processes which could eventually result in fibrogenesis and the development or aggravation of chronic

lung disease. This study did not examine chronic effects, and it is an open question as to whether human populations with high ambient exposures to PM₁₀ might exhibit decreased lung compliance and possibly decreased pulmonary function as measured by forced expiratory maneuvers. While some epidemiology findings might support this contention, the specific role played by PM₁₀ as compared to that played by oxidant gases such as ozone is not clear since acute ozone exposure can cause many of these same effects, and the same populations might be exposed to both high PM₁₀ and high ozone concentrations (albeit not necessarily at the same time).

The permeability data which are shown in Figure 4 demonstrate a "batch" effect. These data represent two independent studies, one with high concentration atmospheres and one with low concentration atmospheres which used batches of rats purchased at different times. The protein concentrations measured in the high concentration study were higher overall than those in the low concentration study. In each study, the statistical comparisons are with that studies control group which was derived from the same batch of rats, treated in an identical fashion to the atmosphere groups and sacrificed on the same day. The increase in lung permeability observed in this study suggests that following exposures to PM₁₀ components plasma or serum factors could enter the lung, infiltrating through disrupted tight junctions between epithelial cells (Bhalla et al., 1986). It is not established whether the tight junction disruption is due to changes in the characteristics or receptors for specific cell adhesion molecules, or if it is due to disarrangement of the cytoskeletal structure of these epithelial cells (Bhalla et al., 1990). In extreme cases, this increase in permeability could be related to pulmonary edema, which in individuals with compromised cardiopulmonary systems could be life threatening. Another aspect of this permeability barrier breakdown is that it offers an opportunity for toxic or allergenic materials which are deposited in the lung to infiltrate into the sub-epithelial layers (Bhalla et al, 1988), from which they may be removed much more slowly than they would be from the epithelial surface. This would result in increased contact time of potentially sensitive cells with compounds that could provoke asthmatic responses in some individuals and increase the risk of toxic, mutagenic or carcinogenic effects. One aspect that could increase the possible importance of permeability changes as a key mechanism for linking PM₁₀ exposure to increased acute mortality would be evidence of age-

related susceptibility. After reviewing the available literature it is not clear that elderly individuals are more susceptible to permeability changes than are healthy younger adults. However, in subjects with interstitial lung disease, increased concentrations of albumin in epithelial lining fluid was related to increased age (Roberts et al., 1993). There is evidence from animal studies that neonates and infants show larger increases in permeability than animal with mature lungs when challenged with histamine (Arakawa et al., 1992) or barotrauma (Adkins et al., 1991), and baseline permeability of the newborn lung is greater than that in more mature lungs (Mills et al., 1991; Adkins et al., 1991; Hutchison et al., 1985). This suggests that neonates and infants may represent a sensitive population subgroup whose response to PM10 exposure should be evaluated.

Macrophages from rats exposed to low concentrations of PM10 components exhibited decreased antibody-directed ability to bind antigenic material to Fc receptors on the macrophage cell membrane, but only SO_4^{2-} provoked a group mean value which was significantly different from that of the control group. We did not observe a significant effect of road dust at either high or low concentration. Ziegler et al. (1994), on the other hand, showed reduced expression of Fc receptors on macrophages from road dust exposed rats, but not nitrate-exposed rats (SO_4^{2-} -exposed rats were not studied). This discrepancy may be due to methodological differences (Ziegler studied fixed cells while this study examined live cells) or may relate to differences in specific Fc receptors. More study will be needed to resolve these differences. The present study also demonstrated that high concentration road dust and sulfate exposures reduced the ability of macrophages to mount a respiratory burst to generate superoxide and presumably other biocidal and bactericidal compounds which are an important part of the lung's defense against inhaled pathogens. It is interesting to note however, that at lower concentrations there was a tendency (which was not statistically significant) for macrophages from PM10 component-exposed rats to generate excess superoxide. Ruiz et al. (1988) have reported that macrophages from children exposed to high environmental levels of airborne particles in Santiago, Chile show increased production of reactive oxygen compounds and concomitantly that there was a reduction of circulating antioxidant compounds, indicative of oxidative stress. Oxidative stress has been linked with the development of cardiopulmonary and cardiovascular diseases. Our findings do

not rule out a mechanistic link between PM10 exposure and the development of heart and lung disease, and suggest a need for additional studies.

This study demonstrated significant changes in lung morphometry, permeability and alveolar macrophage functions following exposures to PM10 components. The most consistent effects were observed in rats exposed to the two water soluble PM10 components, nitrate and sulfate. In terms of potency relative to the concentrations of each component administered, the effects could be ordered as $\text{SO}_4^{-2} > \text{NO}_3^{-1} > \text{road dust}$. Both sulfate and nitrate are predominantly found as submicron particles in ambient PM10, and the sizes generated for this study were 0.2 and 0.6 μm MMAD, respectively. Although in ambient air, road dust is mostly found in the supermicron particle fraction of PM10, there are small but significant contributions of crustal elements from road dust to the submicron particle fraction of PM10 (Ehrman and Pratsinis, 1992). In this study only 4 μm MMAD road dust particles were tested. Further studies with other sizes of road dust particles will be needed before this component can be ruled out from among the possible contributors to the apparent effects of ambient PM10 exposure on human health. The results of this study suggest that the mechanisms responsible for specific airway responses may be different for individual PM10 components. Although the nature of these mechanisms is not yet clear, it appears that the larger particles do not necessarily affect epithelial functions, but may elicit macrophage responses.

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Table 1. Maximum 24 Hr PM10 Concentrations in Southern California Ambient Air (mg/m³)

City (Year)	Sulfate	Nitrate	Crustal	PM10
Azusa (1986)	0.020	0.060	--	0.183
Rubidoux (1988)	0.021	0.093	0.172	0.243
Riverside (1988)	0.017	0.092	0.151	0.202
Size Range (µm)	0.2-0.5	0.4-0.8	4-5	≤ 10

Study Concentrations			
Low	0.020	0.090	0.300*
High	0.070	0.350	0.900
MMAD (µm)	0.2	0.6	4

*Road Dust used as surrogate

Figure 1a. Alveolar Wall Changes
(Mean \pm SE)

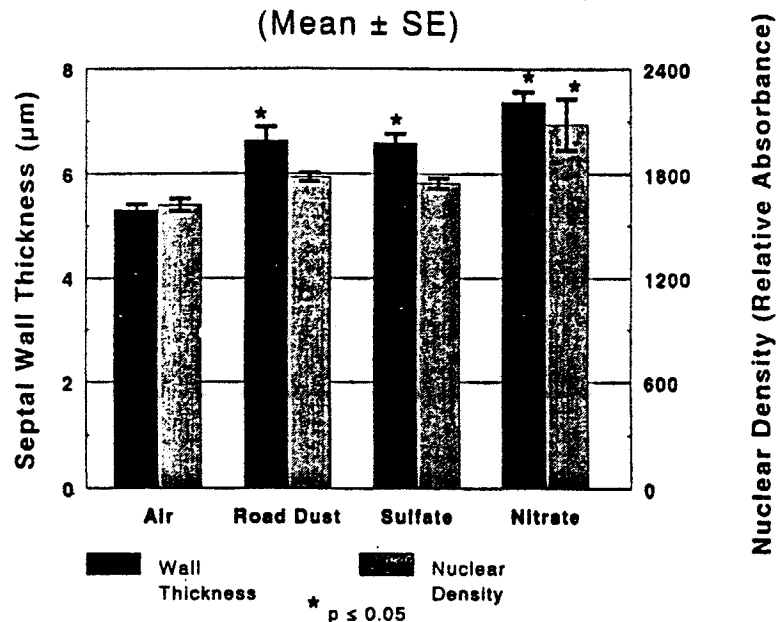
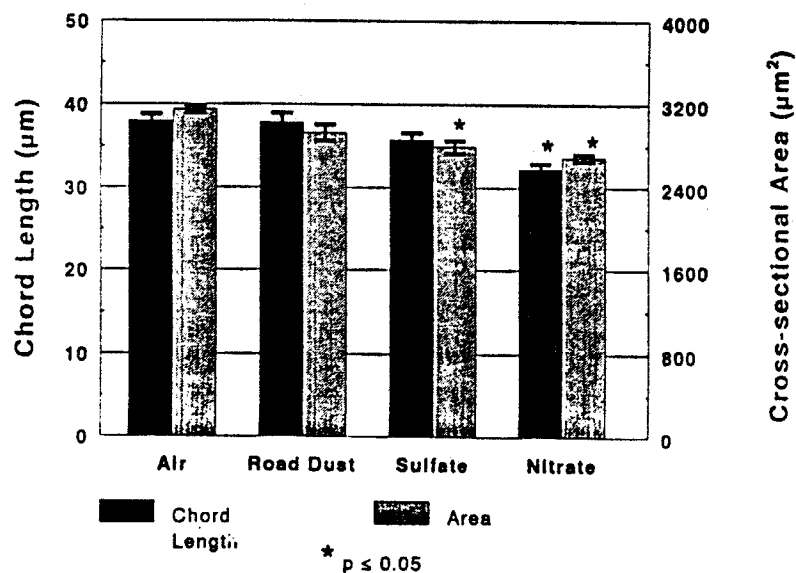


Figure 1b. Alveolar Size
(Mean \pm SE)



- Figure 1.
- Changes in alveolar wall thickness and alveolar wall cellularity, as measured by nuclear density, following exposure to road dust ($900 \mu\text{g}/\text{m}^3$), ammonium sulfate ($70 \mu\text{g}/\text{m}^3$), and ammonium nitrate ($350 \mu\text{g}/\text{m}^3$).
 - Changes in alveolar chord length and alveolar cross-sectional area following exposure to road dust ($900 \mu\text{g}/\text{m}^3$), ammonium sulfate ($70 \mu\text{g}/\text{m}^3$), and ammonium nitrate ($350 \mu\text{g}/\text{m}^3$).

Figure 2. Macrophage FcR Activity
(Mean \pm SE)

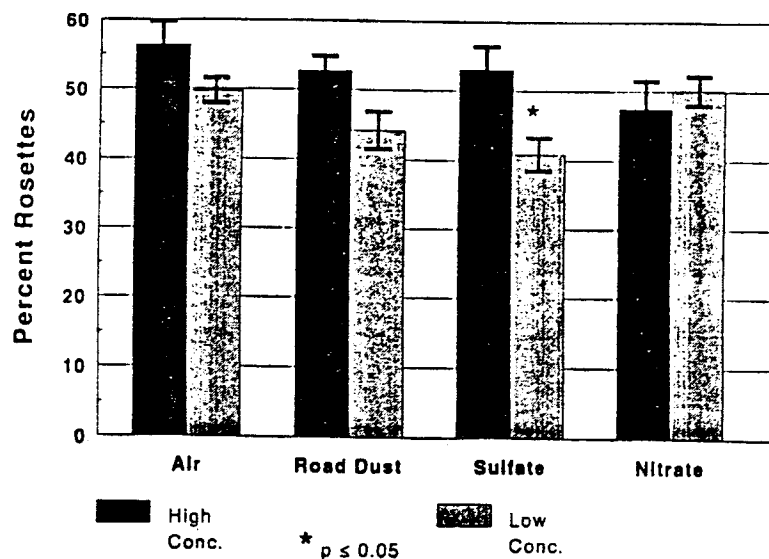


Figure 2. Changes in macrophage Fc receptor binding activity after exposure to PM₁₀ components at high and low concentrations: road dust, 900 or 300 $\mu\text{g}/\text{m}^3$; ammonium sulfate 70 or 20 $\mu\text{g}/\text{m}^3$; and ammonium nitrate 350 or 90 $\mu\text{g}/\text{m}^3$

Figure 3. Macrophage Respiratory Burst
(Mean \pm SE)

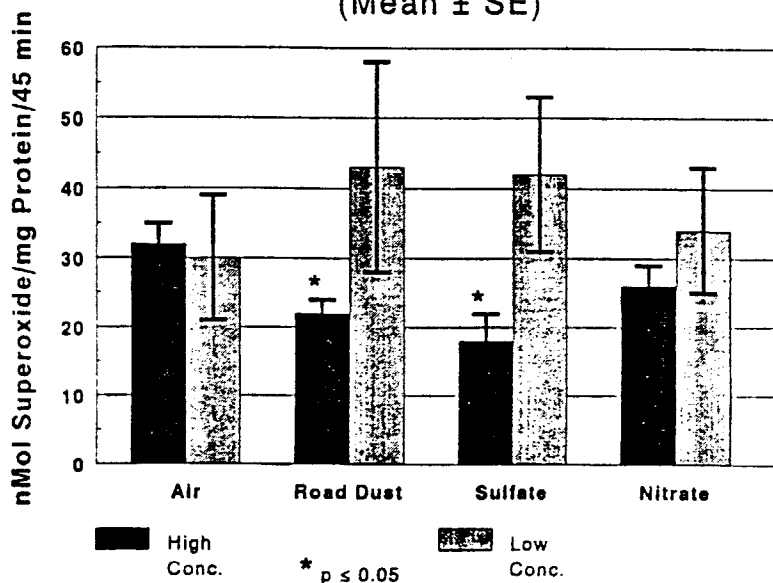


Figure 3. Production of superoxide anion during respiratory burst activity after exposure to PM₁₀ components at high and low concentrations: road dust, 900 or 300 $\mu\text{g}/\text{m}^3$; ammonium sulfate 70 or 20 $\mu\text{g}/\text{m}^3$; and ammonium nitrate 350 or 90 $\mu\text{g}/\text{m}^3$

Figure 4. Airway Permeability
(Mean \pm SE)

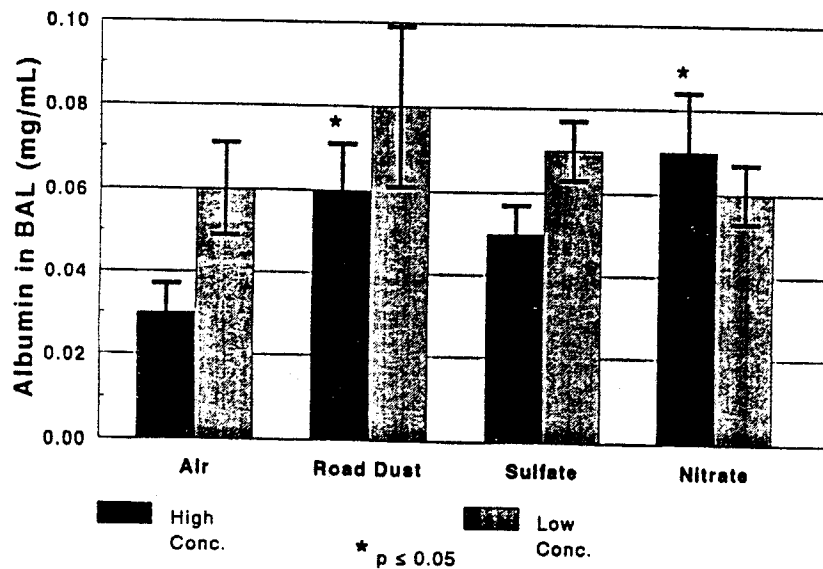


Figure 4. Changes in epithelial permeability as measured by the concentration of albumin in bronchoalveolar lavage fluid after exposure to PM10 components at high and low concentrations: road dust, 900 or 300 $\mu\text{g}/\text{m}^3$; ammonium sulfate 70 or 20 $\mu\text{g}/\text{m}^3$; and ammonium nitrate 350 or 90 $\mu\text{g}/\text{m}^3$.

Dosimetric Issues Relating to Particulate Toxicity*

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ABSTRACT

An increasing number of epidemiological studies have reported excess mortality and morbidity thought to be associated with elevated levels of particulate matter air pollution. These studies call into question the adequacy of the current National Ambient Air Quality Standard for Particulate Matter as being protective of human health. The lack of data from the animal toxicology literature supportive of the types of effects seen in the epidemiology studies has raised issues of biological plausibility, adequacy of animal models, and relevance of endpoints measured in these models. We focused on various aspects of interspecies differences (rat vs. human) in the dosimetry of particles that may help explain the apparent lack of consistency between the toxicological and epidemiological findings. We adjusted the predicted thoracic deposition fractions in rats for the probability of inhaling particles up to 10 μm in diameter. While deposition of particles on a mass per unit alveolar surface area is not different between these species, dose metrics based upon particle number per various anatomical parameters (ventilatory unit, alveolus, or alveolar macrophage) exhibit some striking differences between rats and humans. This is particularly the case for particles 0.1 μm to 0.3 μm in size (i.e., those in the condensation mode of atmospheric aerosol fine particles). Particle deposition studies in smokers and in subjects with lung diseases, such as asthma and chronic obstructive pulmonary disease, show that these subpopulations are likely to be at increased risk from exposure to particulate air pollution. For dose metrics based upon particle number per anatomical parameter, we found that the trend of differences between rats and humans was even more pronounced for these "compromised lung" individuals compared to "normal" subjects. We hypothesize that "localized overload" of particulate clearance mechanisms in individuals with compromised lung status may be part of the biological plausibility story, and we examine various dosimetry model predictions and dose metrics that point in this direction. While our analyses and conclusions should be currently viewed

as preliminary and speculative in nature, they underscore the need for additional research to identify and understand the role of factors leading to acute mortality and morbidity associated with episodic particulate air pollution excursions.

INTRODUCTION

Dust standards are established by regulatory and advisory groups for worker protection. Also, regulatory agencies set standards for particulate matter exposure in order to protect the general public. Recent epidemiology studies of acute mortality have raised concerns that the current National Ambient Air Quality Standard (NAAQS) for particulate matter (PM-10) may not be protective of human health. Schwartz (1994) has reviewed the data base and conducted a meta-analysis. A key finding of this analysis is the lack of a threshold for the relationship between increased mortality and ambient levels of particulate matter. In contrast, there does not appear to be an analogous finding in the animal toxicology literature, even for exposures orders of magnitude higher than those associated with effects in humans.

Dockery and Pope (1994) have reviewed acute morbidity and particulate air pollution studies in the literature, evaluating them for concordance and consistency. Increased hospital admissions, decrements in lung function, and increased reporting of respiratory symptoms were all correlated with PM-10 levels. Increased bronchitis in children has been shown to relate to PM-15 levels in the six-city studies (Speizer, 1989). However, the data on increased bronchitis in children also show a relatively strong relationship with the amount of hydrogen ion (H^+) in the air of these cities (U.S. EPA, 1989). Based upon these findings and upon various effects of acidic aerosols in animals, such as increased airway hyperreactivity, alterations in clearance, and effects on alveolar macrophages (Schlesinger, 1990), one might reasonably ask whether we are focusing too quickly on PM-10 as the most appropriate indicator of toxicity or

whether a dose metric based upon H^+ might be in order. With biological plausibility lacking from a toxicological perspective, Utell and Samet (1993) urge caution in interpreting the current epidemiological findings in the absence of additional toxicological research that would underpin the epidemiology studies.

Much of the debate about the apparent lack of consistency between the epidemiological findings and animal toxicology has focused on the appropriateness of the animal models and the endpoints studied using these models. Inherent in this perspective is an assumption that issues relating to species sensitivity are the basis for the observed difference. However, species sensitivity (i.e., lack of equivalency of biological response for the same dose delivered to the target site in different species) is only part of the picture. Interspecies differences in the dosimetry of particles may also be part of the reason for the apparent lack of agreement.

In this paper, we discuss some dosimetry issues that may explain differences observed between animal and human responses to particulate exposures. Our starting point is the question: Do the available data on particle deposition in laboratory animals and humans provide any insights into the discrepancy in effects seen and do they provide any pointers on where to look for the answers? Among the topics examined are: 1) What are the major similarities and differences between animals and humans in the deposition of particles? 2) Is there a subrange of ambient particles likely to be responsible for the epidemiological effects? 3) Is deposition increased in specific subpopulations and are they more susceptible to particulate matter exposure? We hypothesize that "localized overload" of particulate clearance mechanisms may be a part of the biological plausibility story, especially involving individuals with compromised lung status. Data from the literature are synthesized and deposition

calculations using dosimetry models are presented to address the above questions and our hypothesis.

DEPOSITION OF PARTICLES

Particles depositing in the tracheobronchial or pulmonary regions constitute thoracic deposition. For various laboratory animals and humans, Panel A of Figure 1 shows the predicted thoracic deposition of particles as a function of aerodynamic diameter (d_{ae}). Subsequent references to particle size will use aerodynamic diameter. Separate deposition curves are shown for oral and for nasal breathing in humans, since the route of breathing has a large effect upon the location and extent of thoracic deposition in humans (Miller et al., 1988; U.S. EPA, 1982, 1986). The equations used to predict the animal deposition curves were developed from experimental measurements made by Raabe and colleagues (1988). Thoracic deposition of particles larger than $5 \mu\text{m } d_{ae}$ is small in the nonhuman animal species shown. Although not shown, there is significant information on the thoracic deposition of particles between 0.1 and $1 \mu\text{m}$ in humans (Chan and Lippmann, 1980; Stahlhofen et al., 1986; Schiller et al., 1988). Only limited data are available for deposition in animals of particles between 0.1 and $1 \mu\text{m}$ (Raabe et al., 1988); however, thoracic deposition patterns for particles in this interval do not vary significantly between species. Ultrafine particles (i.e., those with physical diameters $< 0.1 \mu\text{m}$) have not been well studied in experimental animals, and only limited data are available for humans (Schiller et al., 1988).

In most experimental deposition studies, all particles in the test aerosol are tacitly assumed to be inhaled by the subject, since they are usually delivered by a mouthpiece. However, in the real world, humans and animals inhale particles from the air space proximal to the mouth. The relationship between particle concentration in

this air and the more distal ambient air is not necessarily one of identity. Flow velocity patterns around an individual's or animal's head and body and the inertia of the particles can influence the penetration of ambient particles into the breathing zone. Inhalability refers to the probability that a particle of a given size will actually enter the respiratory tract. When multiplied by the ambient concentration, the result is the concentration of particles in the inspired air. Inhalability of different particle sizes varies between species and is an issue that must be addressed when comparing human and animal deposition data. While it is generally agreed that inhalability must decrease as a function of particle size, there is no consensus on what the exact relationship should be and very little data are in the literature on this topic. The American Conference of Governmental Industrial Hygienists (1992) has developed a convex function with a lower bound of 0.5 (i.e., 50%) for inhalability of particles of up to 100 μm in diameter based on studies in mannequins in wind tunnels. Breyse and Swift (1990) developed a concave curve using experimental measurements in four human subjects breathing in still air. Their curve predicts that no particles greater than about 40 μm in diameter can be inhaled.

Using the logistic function, the Breyse and Swift (1990) data were refit. The resulting equation provides similar predictions to those of Breyse and Swift over most of the range, but also allows a small fraction of the large particles to be inhalable, consistent with other observations. Using radioactive count data underpinning the particle deposition data of Raabe et al. (1988) (personal communication, Ms. Margaret Ménache, Duke University Medical Center, Durham, North Carolina), the animal data were also fit to the logistic function to provide an estimate of inhalability in small laboratory animals. While an overall inhalability curve for common laboratory animals was developed, here we will only focus on rats since the vast majority of particulate toxicity data in the open literature have been obtained using this species. A

comparison of predicted thoracic deposition of particles for rats and humans taking inhalability into account is shown in Figure 1 (Panel B). Ambient concentration appears to be a good predictor of thoracic deposition in humans for particles up to about 11 μm in diameter, the size range of most interest for health risk assessments.

Figure 1 (Panel B) basically shows that inhalability for humans is not an issue as far as ambient particles are concerned, and so no adjustments for inhalability will be made to the human deposition and dosimetric calculations presented here. However, inhalability is an issue for the rat. Inhalability is predicted to decline rapidly in rats and be slightly less than 50% for 10 μm particles. For particle sizes as small as 1 μm , there is reduced inhalability in rats compared to humans, with these differences in inhalability being very pronounced for particles $> 5 \mu\text{m}$. The effect of inhalability on thoracic deposition in rats is most pronounced for particles of about 1 to 5 μm d_{ae} . Particles larger than 5 μm d_{ae} deposit predominantly in the upper respiratory tract of the rat (Raabe et al., 1988). As a result, predicted thoracic deposition for such particles is small even without considering inhalability.

Clearly, if the coarse-mode component of ambient aerosols is responsible for the increased acute mortality and morbidity seen in the epidemiology studies, a significant upward adjustment in experimental exposure concentration would be needed when selecting animal toxicological results for comparison to humans. Moreover, from an interspecies dosimetric extrapolation viewpoint, differences between animals and humans in the inhalability of particles should be taken into account for all particle sizes.

SUBRANGE OF AMBIENT PARTICLES CAUSING EFFECTS?

Is there a particle size subrange within ambient aerosols that could be responsible for the effects seen in epidemiology studies? Since the coarse-mode fraction is mainly crustal material, such as iron, calcium, and silica, and since pulmonary deposition of coarse-mode particles is limited (Miller et al., 1979), a reasonable starting point for identifying a subrange of particles associated with the epidemiologic findings is to look at the fine mode (i.e., particles with $d_{ae} < 2.5 \mu\text{m}$). For example, the PM-2.5 fraction in the San Joaquin valley dominates in the late fall and winter months with a twofold increase compared to the spring and summer months; seventy-five to eighty percent of the PM-2.5 mass is related to this fraction and of that, organic and elemental carbon, nitrate, sulfate, and ammonium clearly predominate (Chow et al., 1993). Chow and coworkers (1993) note that the highest seasonal PM-10 mass is similar between the Los Angeles region and the San Joaquin valley, as are the chemical compositions of these components. Interest in the fine mode is further heightened by the accumulating body of evidence that, while the appearance is one of a single mode, two distinct modes can exist in the 0.1 - 1.0 μm diameter range of atmospheric aerosols (Hering and Friedlander, 1982; Wall et al., 1988; John et al., 1990; Meng and Seinfeld, 1994). The two modes have aerodynamic diameters of about 0.2 and 0.7 μm , respectively (John et al., 1990); the smaller particle size mode is denoted as the condensation mode, while the larger has been termed the droplet mode (Meng and Seinfeld, 1994). If toxicity is being mediated by particle number or number per surface area rather than on a mass basis, then characterizing the composition and toxicity of particles in the condensation mode may be particularly important for understanding the epidemiological studies.

Given the number of locations, both within the United States and in other countries, in which acute mortality and morbidity have been reported in epidemiology studies,

should we be looking to the fine mode of ambient aerosols as a unifying factor? And, if so, how do laboratory animals and humans compare in the deposition of these particles?

Predicted thoracic deposition fractions, as a function of airway generation, for selected particles ranging from $0.01\ \mu\text{m}$ (physical diameter) up to $5\ \mu\text{m}\ d_{ae}$ are shown in Figure 2 for humans and in Figure 3 for rats. The computations for humans were made using a particle dosimetry model (Gerrity et al., 1979) based upon the symmetric, single-path lung structure of Weibel (1963).

Oral breathing at 30 breaths per minute with a tidal volume of 500 ml was used for the human dosimetry calculations, thereby approximating a "worst case" scenario. This scenario would tend to minimize extrathoracic removal of particles, thereby increasing thoracic deposition. Also, rapid shallow breathing can be expected during increased activity in many individuals with COPD since their resting breathing frequencies are 20-23 breaths per minute and their tidal volumes are 450-480 ml (Tobin et al., 1983). The dosimetry calculations for the rat were obtained from a multiple-path asymmetric lung model (Anjilvel and Asgharian, 1994) using tracheobronchial airway data from Raabe et al. (1976) and pulmonary acinar data from Yeh et al. (1979).

Predicted deposition in the rat lung for both the tracheobronchial and pulmonary compartments agreed well with the experimental data of Raabe et al. (1977), as well as with the theoretical calculations of Schum and Yeh (1980), who used a single-path model of the rat lung. Except for the ultrafine particle size ($0.01\ \mu\text{m}$), the overall pattern for fractional deposition is reasonably similar between rats and humans for fine-mode particles ($< 2.5\ \mu\text{m}\ d_{ae}$). The deposition of coarse-mode particles is considerably less in rats than in humans. This is due to airway geometry and

ventilatory flow rate differences between the two species that affect particle loss by the primary mechanisms of deposition (impaction, sedimentation, and diffusion).

When these deposition fractions are used for a scenario of 24 hours of exposure to the daily average NAAQS for PM-10 ($150 \mu\text{g}/\text{m}^3$), the mass deposition fraction per unit surface area can be calculated for each airway generation (Figures 4 and 5). The mass deposition patterns are similar between the two species for the 0.1 and 1 μm particles. For the largest particle size shown (5 μm d_{ae}), deposition in the human tracheobronchial airways increases in the first few generations and then falls monotonically. The 5 μm curve for the rat is much lower than for humans and is also broader. While predicted deposition for 0.01 μm particles is shown in Figures 4 and 5, a concentration of $150 \mu\text{g}/\text{m}^3$ of this size particle could not exist for more than a few seconds due to coagulation based on the high number density. (For example, in 4 seconds, the average diameter of particles is 0.086 μm , and it is 0.15 μm in a half-hour.) However, we include this scenario because it is illustrative of the influence of particle diameter on deposition. Although the mass deposition for this size particle would be much lower in both humans and rats exposed to an actual ambient aerosol, there is still a large number of ultrafine particles present in such aerosols.

What do the data in Figures 2-5 tell us about the apparent discrepancies between the acute epidemiological and animal toxicological effects? Many of the epidemiology effects involve individuals with chronic obstructive pulmonary disease (COPD) or with asthma. A comparison of alveolar mass burden differences between rats and humans is given in Table 1 for 0.1, 1 and 5 μm particles. The ratio of mass of deposited particles per unit surface area of the human lung, divided by the mass per unit area received by rats, is given in the last column of Table 1. For the range of particles that would comprise approximately 95% of the fine-mode fraction of urban aerosols, the

human to rat ratios of 0.22 to 0.64 given in Table 1 indicate that rats receive a mass burden from about 1.5 to 4.5-fold greater than humans. Thus, one would expect that the animal toxicological studies should be able to demonstrate effects comparable to those seen in humans, if mass per unit area is the driving dose metric and if the animal model being used and endpoint being measured are appropriate indicators of human responses. Yet the vast amount of toxicological data, with a few exceptions, does not show comparability to the human results.

DEPOSITION IN SPECIFIC SUBPOPULATIONS

The literature is replete with experimental deposition studies using healthy, nonsmoking adults, with much of this information available in reviews (Lippmann, 1977; Lippmann et al., 1980; U.S. EPA, 1982, 1986; Schlesinger, 1985, Stahlhofen et al., 1989). Most of these data were used to develop the model used in the prediction of the human deposition fraction curves shown in Figure 1. Particle deposition has been experimentally measured in smokers, and enhanced tracheobronchial deposition was found over a wide range of particle sizes (NRC, 1977). A family of deposition curves predicting the increase in particulate deposition in smokers compared to nonsmokers was developed by the NRC (1977). In addition to increased deposition in this subpopulation, inhaled particles may possibly be retained in the conducting airways for a longer period of time (Stahlhofen et al., 1989), thereby increasing the potential for the particles to exert biological effects. Clearly, including smoking status as an independent variable is an important feature of well conducted epidemiological investigations of the effects of air pollutants on acute mortality and morbidity.

Various occupational groups receive elevated exposures to particulate matter in the workplace. While experimental data on the deposition of particles in the lung are

primarily from studies using healthy, nonsmoking subjects, limited data are available from deposition experiments using specific occupational groups or subjects with pre-existing pulmonary disease. For example, Stahlhofen (1986) conducted deposition studies using dental technicians; for these individuals, exposure to silica and certain heavy metals are potential occupational hazards. Stahlhofen (1986) found that dental technicians with more than ten years of work history have a smaller ratio of alveolar to thoracic deposition of $5\ \mu\text{m}$ particles compared to controls, resulting from a decrease in the alveolar deposition fraction accompanied by an increase in tracheobronchial deposition. This difference in ratios is even more pronounced in dental technicians with bronchitic symptoms.

Kim and coworkers (1988) examined aerosol deposition in subjects with various lung conditions: normals, asymptomatic smokers, smokers with small airway disease, chronic simple bronchitis, and chronic obstructive bronchitis. They found a significant gradient between these groups relative to the number of breaths required to deplete a $1\ \mu\text{m}\ d_{ae}$ test aerosol of 90% of its particles (N_{90}) during aerosol rebreathing. The N_{90} for chronic obstructive bronchitic subjects averaged 3.8 breaths compared to 10.5 breaths in normal subjects, reflecting a much more rapid deposition of the test aerosol in the subjects with compromised lungs compared to those with healthy lungs. More rapid deposition in this clinical testing procedure can be interpreted to mean that such individuals will have increased deposition compared to normal subjects in any given particulate exposure scenario.

There is a mismatch of ventilation and perfusion in lung diseases, such as asthma, emphysema, and COPD (Bates et al., 1971, Bates, 1989). In more severe stages of these diseases, a small portion of the lung volume receives most of the tidal breathing volume. Bates et al. (1971) notes that only one-fourth of the lung receives about three-

fourths of the tidal volume in patients with emphysema. This will result in some ventilatory units receiving a greatly increased particle burden compared to others. Here, a ventilatory unit is defined as the collection of all alveoli distal to a given terminal bronchiole. Additionally, a major feature of asthma is that there is a mismatch of ventilation and perfusion even when pulmonary function (as evidenced by the amount of air that can be forcibly exhaled in one sec) has returned to normal (Bates et al., 1989). Without additional research in individuals with compromised lung function, the inference that local deposition gradients may initiate a cascade of events leading to the acute morbidity and mortality effects seen in the epidemiology studies remains speculative, but plausible. More importantly, there is a need to develop animal models of lung disease that can be used to identify the mechanistic linkages in the sequelae of events leading to acute mortality and morbidity from exposure to elevated levels of particulate air pollution.

LOCALIZED OVERLOAD OF PARTICLES

How could highly localized excess burdens of particulate matter develop in the lung? Consider this sequence. Over a prolonged period of time, peripheral deposition of particles becomes nonuniform as a function of the size and composition of inhaled particles, coupled with an individual's specific tracheobronchial branching structure and existing damage pattern. With continuing deposition, some ventilatory units become more and more compromised due to some combination of disease and particle-induced damage. This may progress in individuals with COPD to the point where most of the inspired air goes to only a small portion of the lung volume. Then when an episodic excursion of particulate pollution occurs, there is an increased probability of localized overloading of ventilatory units and a triggering of a cascade of inflammatory and other events that could severely limit the ability of the lung to satisfy

the ventilatory needs of an individual, thereby leading to enhanced morbidity or death depending upon the individual's reserve capacity.

In recent toxicological literature, the term "overload" has referred to the overwhelming of the ability of alveolar macrophages to phagocytize and remove particles from the lung and has been demonstrated primarily in Fischer 344 rats. Morrow (1992) suggests that the overload phenomenon is linked to the volume of particles ingested by macrophages and a resultant cascade of recurrent inflammatory events as the most likely explanation in view of currently available data. However, Morrow (1992) notes the need for examining particle overload in nonrodent species and the particular need for more information on the role of the alveolar macrophage in particle removal in the human lung.

Having examined the mass burden ratio between rats and humans in an earlier section of this paper, we now examine alternative dose metrics based upon particle number for potential support of localized overload being a factor leading to the observed acute epidemiological effects. For exploring potential particle-related dose metrics, pertinent ventilatory and structural data we used in our analyses are given in Table 2 for rats and humans. There are about 2,500 acini (ventilatory units) in the rat lung (Yeh et al., 1979), compared to about 33,000 such units in the human lung (i.e., essentially the number of terminal bronchioles in the Weibel (1963) lung). Estimates of the number of alveoli in the rat range from 19.7 million (Stone et al., 1992) to 30 million (Yeh et al., 1979). For humans, Weibel (1963) gives the number of alveoli as 300 million, while Stone et al. (1992) report this number to be 494 million. The number of macrophages per alveolus is 1.5 in the rat compared to 12 in the human lung (Stone et al., 1992).

In examining possible dose metrics, a specific exposure scenario is used that corresponds to 24 hr exposure to $150 \mu\text{g}/\text{m}^3$ (the daily NAAQS for PM-10). Dosimetry models for the rat and human were used to compute the mass predicted to be deposited in the alveolar region of these species for monodisperse aerosols of various sizes. To obtain particle deposition estimates for the compromised lung, the deposition associated with 75% of the tidal volume was apportioned to one-fourth of the ventilatory units, along with a corresponding adjustment in the number of alveoli associated with these units. Given that specific tracheobronchial paths, as well as different modes of breathing and lung filling (Chang, 1984), would need to be examined to approach the problem more directly for individuals with compromised lungs, the above allocation was viewed as a reasonable first approximation. Assuming a particle mass density of $1 \text{ g}/\text{cm}^3$ and using the data in Table 2, conversion of deposited mass to deposition values based upon particle number is possible. Various expressions for dose are given in Table 3 for 0.1, 1, and $5 \mu\text{m}$ particles.

As with mass deposition of particles, the various dose metrics in Table 3 based upon particle number indicate that large particles (greater than about $4\text{-}5 \mu\text{m } d_{ae}$) are not likely to be responsible for the acute epidemiological effects, in normal individuals or in those with compromised lungs. For example, there are only 2 to 7 particles per 1000 macrophages in the human lung and only 3 to 9 particles per 100 alveoli. Even for $1 \mu\text{m } d_{ae}$ particles, there is an average of only one particle per alveolus associated with exposure of humans to $150 \mu\text{g}/\text{m}^3$ for 24 hours. However, very small particles ($0.1 \mu\text{m}$) result in significant numbers of particles per alveolus (1930) and per alveolar macrophage (161) in the normal human lung; while in the compromised human lung, the corresponding numbers are 5,790 and 482, respectively. This represents approximately a 3-fold increase over the normal lung for both dose metrics. Moreover, compared to a rat breathing the same $150 \mu\text{g}/\text{m}^3$ (see Table 3), an individual with

severe COPD receives about 25-fold more $0.1\ \mu\text{m}$ particles per alveolus and 3-fold more per macrophage than does a rat (i.e., a rat would have to be exposed to $3,750\ \mu\text{g}/\text{m}^3$ to receive the same number of particles per alveolus and to $450\ \mu\text{g}/\text{m}^3$ to get the same number per macrophage).

Figure 6 depicts the relationship between the predicted number of particles per alveolar macrophage and particle diameter for particles ranging in size from $0.1\ \mu\text{m}$ to $10\ \mu\text{m}$. The number of particles per macrophage is significantly elevated for all particle sizes in individuals with compromised lungs compared to normal individuals. Exposure to particles in the condensation mode of urban aerosols results in lung burdens of hundreds of particles per macrophage. While 482 particles of $0.1\ \mu\text{m}$ size per alveolar macrophage does not meet an "overload" criterion based upon a critical ingestion volume of about 6% of the total cell volume (Morrow, 1988, 1992), it is plausible that the cell's metabolic and/or functional machinery may not be able to handle hundreds of repetitive actions in a relatively short time span. Thus, localized overload based upon number and inherent toxicity of the particles may well be a viable explanation for the recent findings in epidemiology studies.

The above calculations are for monodisperse aerosols. The total number of particles predicted to deposit when breathing polydisperse aerosols of the same mass median aerodynamic diameter (MMAD) with geometric standard deviations (σ_g) from 1.8 - 2.0 μm tends to be greater than the number deposited when breathing monodisperse aerosols. For example, the predicted number of $1\ \mu\text{m}$ MMAD ($\sigma_g = 1.8$) particles deposited in the alveolar region of the rat lung is 1.3×10^7 , as compared to 1.4×10^6 particles (Table 3) if the aerosol is monodisperse; the predicted alveolar deposition of $5\ \mu\text{m}$ MMAD ($\sigma_g = 2$) particles is 1.3×10^5 for the rat, but is 1.6×10^4 for $5\ \mu\text{m}$ monodisperse particles. Given the overall uncertainties and the myriad of possible

scenarios that could be computed, an assumption of monodisperse aerosols was felt to be reasonable for the intent of the current paper. An additional point worth noting is that the asymmetric lung model of the rat predicts nonuniform deposition of particles in ventilatory units for both very small particles ($0.1\ \mu\text{m}$) and very large particles ($5\ \mu\text{m}$ d_{ae}) (Asgharian and Anjilvel, unpublished observation). Thus, our simplified calculations are likely to underestimate the actual variability in particle dosimetry.

Other dose metrics should also be explored for explanation of the epidemiologic findings. For example, very fine particles can be taken up by other types of alveolar cells, incorporated into the interstitium, and cleared via various pathways. Also, deposition of particles in other regions, such as the tracheobronchial airways, may be important for morbidity outcomes, such as increased hospital admissions in asthmatics. In this case, the number of particles per unit surface area of conducting airways may be a relevant dose metric, and the focus may need to be on larger particles ($> 4\text{-}5\ \mu\text{m}$ d_{ae}). Hence, that both fine and coarse mode particles may be playing a role in acute morbidity should not be dismissed as a possibility.

SUMMARY

Recent epidemiology studies have raised concerns that the current NAAQS for particulate matter is not protective of human health. In contrast, the animal toxicological studies do not appear to be in agreement with these findings, leading to debate over the appropriateness of the animal studies and the biological plausibility currently lacking for the epidemiological findings. We examined the role of particle dosimetry in these matters. Based upon mass deposition per unit area, current dosimetry model predictions do not support a major difference in the dose delivered to the alveolar region of rats compared to humans. However, dose metrics based upon particle number per various anatomical parameters indicate a need to examine the

role of fine-mode particles in the range of 0.1 to 0.3 μm in eliciting acute morbidity and mortality in individuals with compromised lung status, such as patients with COPD. A reasonable argument can be made that, for some individuals, the inhalation of high concentrations of particles between about 0.1 to 0.3 μm may result in a localized overloading of the lung's clearance mechanisms that triggers a cascade of events leading to acute morbidity and/or mortality. However, tracheobronchial deposition of coarse mode particles should not be dismissed as potentially contributing to the acute morbidity seen in asthmatics.

Additional research is needed to identify and understand the role of the factors leading to acute mortality and morbidity associated with episodic particulate air pollution excursions. Some specific dosimetry-related questions such research should address are, for example: 1) How heterogeneous is increased deposition of particles in COPD patients?, 2) Is the time for clearance of particles in asthmatics and persons with COPD longer in comparison with normal subjects, thereby increasing the time period for particles to exert toxicity?, 3) Relative to the rat, do other animal species compare better with humans relative to dose metrics based upon particle number?, and 4) What experimental dosimetry studies are needed to establish whether localized lung overload may indeed be a part of the biological plausibility story for the toxicity of particles in humans at low levels of exposure. This latter question can be better addressed if improved animal models of human cardiopulmonary disease are developed.

DISCLAIMER

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Table 1

Species Comparisons of Alveolar Mass Deposition for Various Particle Sizes
for 24 Hour Exposures to 150 $\mu\text{g}/\text{m}^3$

Particle Size (μm)	Human ^a		Rat ^b		Human/Rat ^c
	Mass (μg)	Mass/ Unit Area ($\mu\text{g}/\text{cm}^2$)	Mass (μg)	Mass/ Unit Area ($\mu\text{g}/\text{cm}^2$)	Ratio of Mass/ Unit Area
0.1	303	5.0×10^{-4}	3.74 - 3.76	2.3×10^{-3}	0.22
1	170	2.8×10^{-4}	0.72 - 0.77	$4.4 - 4.7 \times 10^{-4}$	0.59 - 0.64
5	559	9.1×10^{-4}	1.01 - 1.57	$6.2 - 9.5 \times 10^{-3}$	0.96 - 1.49

^a Deposition computed for oral breathing at a tidal volume of 500 ml and a breathing frequency of 30 breaths per minute.

^b Deposition calculations used a tidal volume of 2.1 ml and a breathing frequency of 102 breaths per minute. If an interval is given for a value, the first number incorporates an adjustment for inhalability while the second number does not.

^c An interval reflects ratios derived with and without adjustment for inhalability in the rat, with the larger value being from inhalability adjusted calculations.

Table 2

Ventilatory and Structural Data for Rats and Humans Used in Current Analyses

	<u>Rat</u>	<u>Human</u>
<u>Ventilatory Parameters</u>		
Breaths per Minute	102	30
Tidal Volume, ml	2.1	500
Minute Ventilation, L ⁻¹ min.	0.2142	15
<u>Structure</u>		
Number of Ventilatory Units	2,404 ^a	32,768 ^b
Alveolar Surface Area at FRC, cm ²	1,650 ^c	611,000 ^d
Number of Alveoli, millions	19.7 ^e - 30 ^f	300 ^b - 494 ^e
Number of Macrophages/Alveolus ^e	1.5	12

^a Raabe et al. (1976)

^b Weibel (1963)

^c Using a surface area to volume of 346 cm²/ml (Mercer et al., 1992), this surface area corresponds to a FRC volume of 4.77 ml. The volume at FRC given by Yeh et al. (1979) was used in our analysis. Since these authors had 2,487 ventilatory units in their model, we used a larger (2,487/2,404) volume per ventilatory unit to keep the overall FRC volume the same for the 2,404 ventilatory units attached to the individual tracheobronchial paths in Raabe et al. (1976).

^d Obtained after synthesizing data on body weight, height, and pulmonary function prior to isotropically scaling (Overton et al., 1987) total lung capacity volume to that at FRC (personal communication, Dr. John H. Overton, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina).

^e Stone et al. (1992)

^f Yeh et al. (1979)

Table 3

Species Comparisons of Various Alveolar Dose Metrics as a Function of Particle Size
for 24 Hour Exposures to 150 µg/m³

Particle Size	Dose Metric	Rat ^a	Human Lung Status		Ratio: Human/Rat	
			Normal	Compromised	Normal	Compromised
0.1 µm	No. ^b Deposited	7.2 x 10 ⁹	5.8 x 10 ¹¹	4.3 x 10 ¹¹	81	61
	No./Unit Surface Area	4.3 x 10 ⁶	9.5 x 10 ⁵	2.8 x 10 ⁶	0.2	0.7
	No./Ventilatory Unit	3.0 x 10 ⁶	1.8 x 10 ⁷	5.3 x 10 ⁷	6	18
	No./Alveolus	238	1,930	5,790	8	24
	No./Macrophage	159	161	482	1	3
1 µm	No. Deposited	1.4 x 10 ⁶	3.3 x 10 ⁸	2.4 x 10 ⁸	237	178
	No./Unit Surface Area	831	532	1,590	0.6	1.9
	No./Ventilatory Unit	570	9,910	29,700	17	52
	No./Alveolus	0.05	1.1	3.25	24	71
	No./Macrophage	0.03	0.09	0.3	3	9
5 µm	No. Deposited	1.6 x 10 ⁴	8.5 x 10 ⁶	6.4 x 10 ⁶	550	413
	No./Unit Surface Area	9	14	42	1.5	4.5
	No./Ventilatory Unit	6	260	780	40	121
	No./Alveolus	0.0005	0.03	0.09	55	165
	No./Macrophage	0.0003	0.002	0.007	7	21

^aRat data values have been adjusted for inhalability

^bNo. = Number of particles

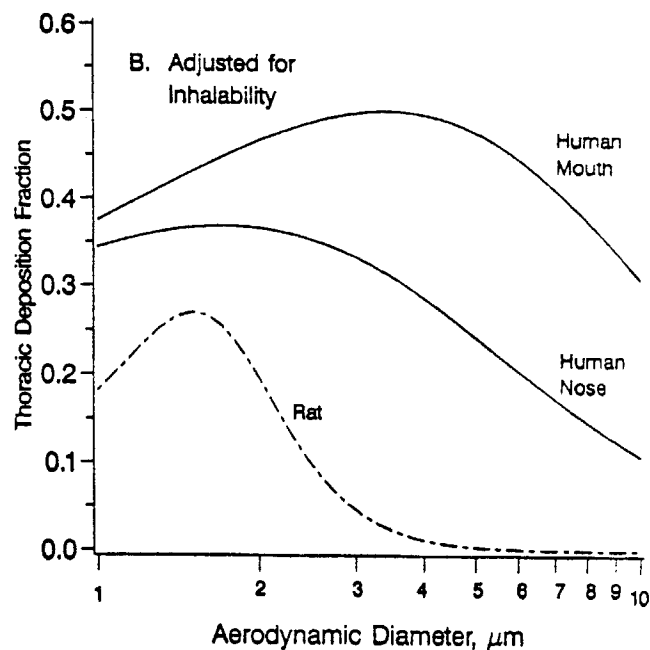
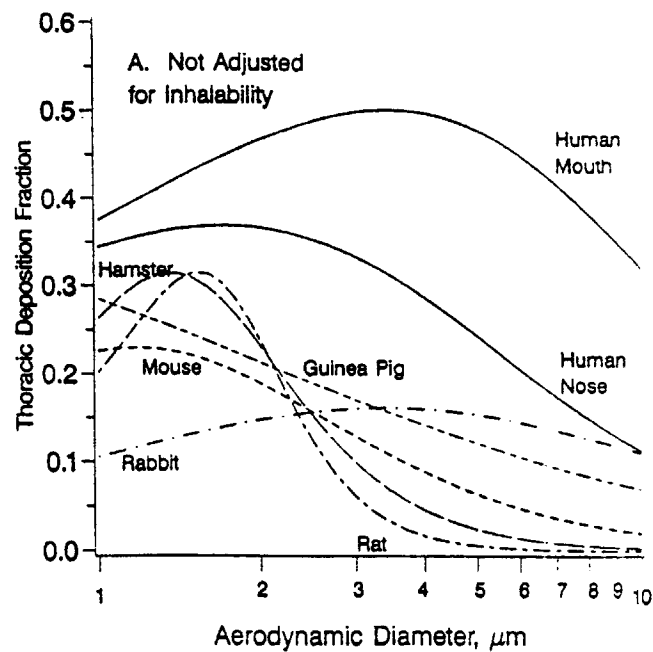


FIGURE 1 Thoracic deposition as a function of the aerodynamic diameter of particles. Deposition was estimated by fitting efficiencies to the logistic function in humans (Miller et al., 1988). The same analytical technique was applied to the animal data of Raabe and colleagues (1988). Panel A shows thoracic deposition for commonly used laboratory animal species and for humans. Separate curves are presented for humans, depending upon whether breathing is through the nose or mouth; the minute volume used was $13.8 \text{ L} \cdot \text{min}^{-1}$. In Panel B, thoracic deposition in rats and in humans is shown after adjusting for the inhalability of particles. The inhalability adjustment was made by multiplying deposition fractions by a logistic function fit to the data of Breyse and Swift (1990) for humans and to the data of Raabe et al. (1988) for rats.

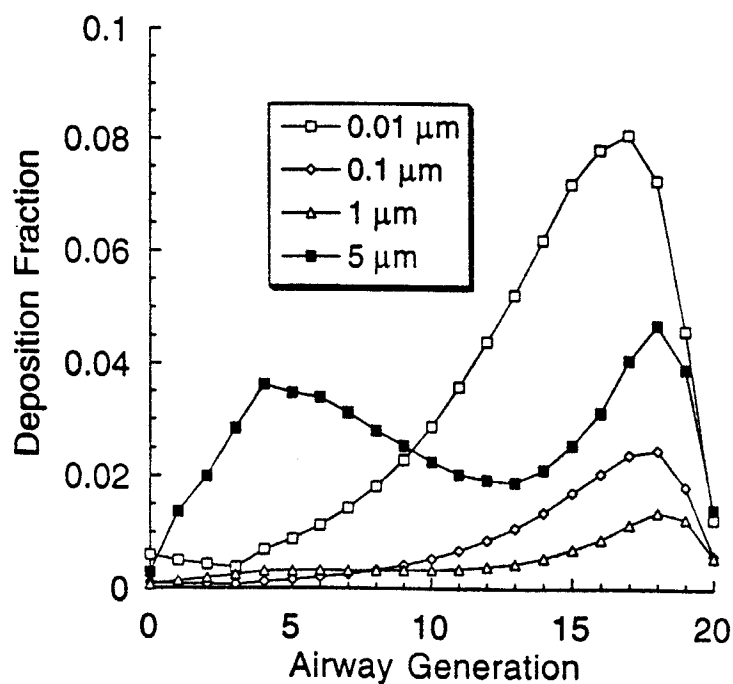


FIGURE 2 Predicted particle deposition fractions in the human lung for various particle sizes as a function of airway generation. The trachea is generation 0, while the alveolated region begins with generation 17. Dosimetry model calculations were for breathing orally at a tidal volume of 500 ml and a breathing frequency of 30 breaths per minute.

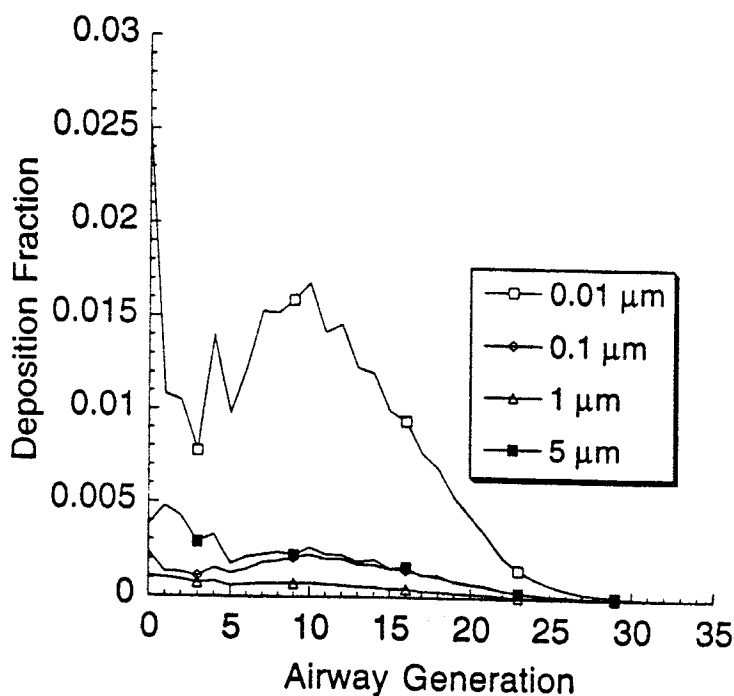


FIGURE 3 Predicted particle deposition fractions in an asymmetric multiple path model of the tracheobronchial airways of the rat lung for various particle sizes as a function of airway generation. The trachea is generation 0. A tidal volume of 2.1 ml and a breathing frequency of 102 breaths per minute were used.

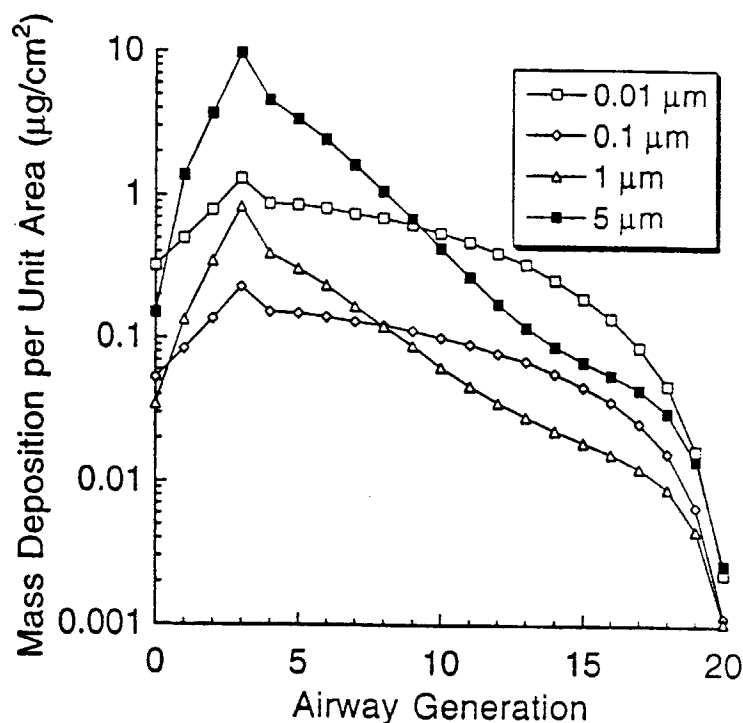


FIGURE 4 The mass of particles per airway unit surface area in the human lung for various particle sizes. Generation 16 is the end of the conducting airways. Model calculations were for 24 hours of exposure to $150 \mu\text{g}/\text{m}^3$ when breathing through the mouth at a tidal volume of 500 ml and a breathing frequency of 30 breaths per minute.

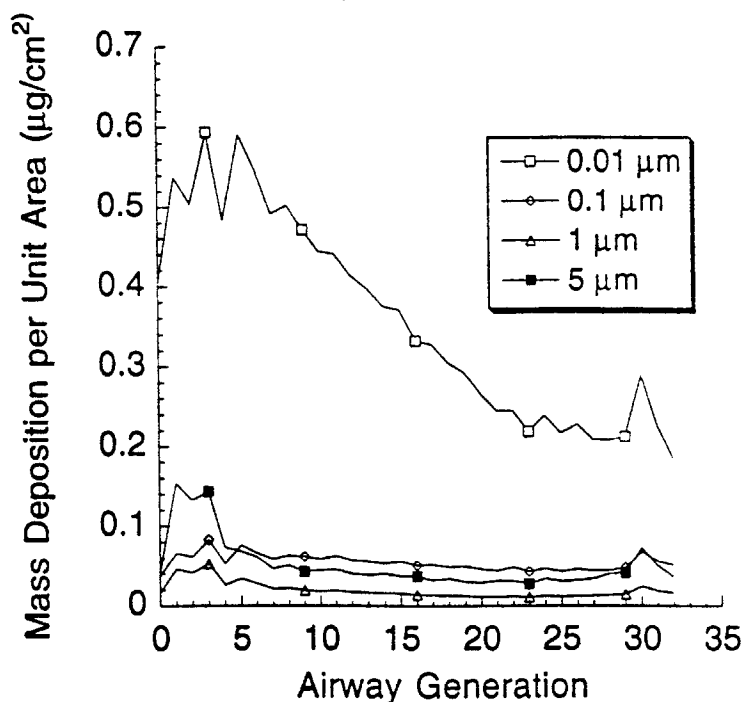


FIGURE 5 Mass of particles per unit surface area in the tracheobronchial airways of the rat lung for various particle sizes. Shown in the figure are the conducting airway doses using an asymmetric multiple path model for the rat, after adjusting for inhalability. Model calculations were for 24 hours of exposure to $150 \mu\text{g}/\text{m}^3$ when breathing through the nose at a tidal volume of 2.1 ml and a breathing frequency of 102 breaths per minute.

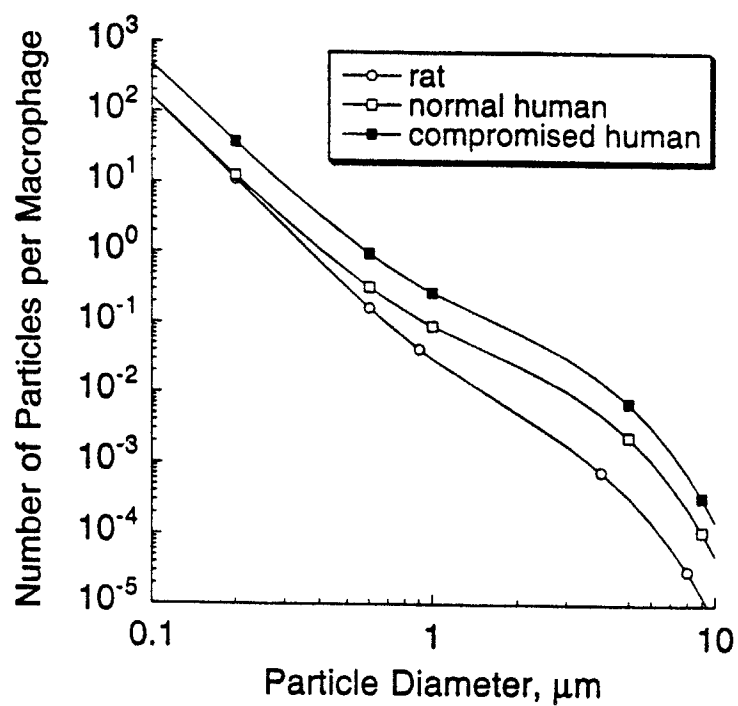


FIGURE 6 Number of particles per alveolar macrophage in humans and rats as a function of particle diameter. For humans, separate curves are presented for normal individuals compared to those with compromised lung status, such as chronic obstructive pulmonary disease.

ASSOCIATION OF PARTICULATE AIR POLLUTION AND ACUTE MORTALITY:
INVOLVEMENT OF ULTRAFINE PARTICLES?

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ABSTRACT:

Recent epidemiological studies show an association between particulate air pollution and acute mortality and morbidity down to ambient particle concentrations below $100 \mu\text{g}/\text{m}^3$. Whether this association also implies a causality between acute health effects and particle exposure at these low levels is unclear at this time; no mechanism is known which would explain such dramatic effects of low ambient particle concentrations. Based on results of our past and most recent inhalation studies with ultrafine particles in rats, we propose that such particles, *i.e.*, particles below ~ 50 nm in diameter, may contribute to the observed increased mortality and morbidity. In the past we have demonstrated that inhalation of highly insoluble particles of low intrinsic toxicity, such as TiO_2 , results in significantly increased pulmonary inflammatory responses when their size is in the ultrafine particle range, *i.e.*, ~ 20 nm in diameter. However, these effects were not of an acute nature and occurred only after prolonged inhalation exposure of the aggregated ultrafine particles at concentrations in the mg/m^3 range. In contrast, in the course of our most recent studies with thermodegradation products of polytetrafluoroethylene (PTFE) we found that freshly generated PTFE fumes containing singlet ultrafine particles (median diameter 26 nm) were highly toxic to rats at inhaled concentrations of $0.7 - 1.0 \times 10^6$ particles per cm^3 , resulting in acute hemorrhagic pulmonary inflammation and death after 10-30 minutes of exposure. We also found that work performance of the rats in a running wheel was severely affected by PTFE fume exposure. These results confirm reports from other laboratories of the highly toxic nature of PTFE fumes which cannot be attributed to gas phase components of these fumes such as HF, carbonylfluoride, or perfluoroisobutylene, or to reactive radicals. The calculated mass concentration of the inhaled ultrafine PTFE particles in our studies was about $64 \mu\text{g}/\text{m}^3$, a very low value to cause mortality. Aging of the fumes with concomitant aggregation of the ultrafine particles significantly decreases their toxicity. Since ultrafine particles are always present in the urban atmosphere, we suggest that they play a role in causing acute lung injury in sensitive parts of the population.

INTRODUCTION:

A number of epidemiological studies conducted over the past five years have demonstrated an association between ambient urban particulate air concentrations down to below $100 \mu\text{g}/\text{m}^3$ and (i) increased mortality, occurring in elderly people with pre-existing cardiorespiratory diseases, (ii) increased morbidity due to respiratory symptoms in children (Schwartz *et al.*, 1991; Schwartz and Dockery, 1992a,b; Dockery *et al.*, 1993; Pope *et al.*, 1991). Although this association has been well-established, there is presently no plausible hypothesis or mechanism which would explain such acute effects induced by these low particulate concentrations and, accordingly, causality has not been proven. It appears that the fine particle phase (PM 2.5) correlates best with the acute mortality and morbidity findings. However, no toxicological studies with particles in the size range around $1\text{-}2 \mu\text{m}$ and at concentrations below $100 \mu\text{g}/\text{m}^3$ are known which would explain these effects unless highly toxic compounds are involved, which are not normal constituents of urban aerosols.

Measurements of ambient aerosol distributions showed a typical trimodal pattern as pointed out by Wilson *et al.* (1977) in their road test studies (Fig. 1). In addition to coarse and fine particles they described an ultrafine particle mode with particles around 20 nm in diameter which was attributed at the time to sulphate aerosols generated from catalytic converters in cars; however, particles from other combustion processes fall into this size range which is not very persistent because these particles tend to agglomerate and then contribute to the next larger particle mode. We hypothesize that this ultrafine particle mode may be significantly involved in the observed acute epidemiological effects. In particular, we suggest that even low mass concentrations of inhaled singlet ultrafine particles ($<50 \text{ nm}$) have a high pulmonary toxicity because of the large numbers of particles involved. This hypothesis is based on (i) results with inhaled freshly-generated fumes which consist of ultrafine particles and lead to the well-known effects of fume fever (metal fume fever, polymer fume fever [Drinker *et al.*, 1927; Makulova, 1965; Rosenstock and Cullen, 1986; Goldstein *et al.*, 1987; Gordon *et al.*, 1992; Blanc *et al.*, 1991, 1993), and (ii) on our previous

studies with ultrafine TiO_2 and Al_2O_3 particles of ~ 20 nm in diameter (Ferin *et al.*, 1992; Oberdörster *et al.*, 1992; 1994). These studies have shown that, compared to larger particles (>0.2 μm) of the same material, ultrafine particles cause a greater inflammatory response in the lungs of rats, including the alveoli and the interstitium (Oberdörster *et al.*, 1992); that they translocate into the pulmonary interstitium more readily, both *via* Type I and Type II cells (Lehnert *et al.*, 1993a; Ferin *et al.*, 1992); that they induce greater activation of alveolar macrophages in terms of cytokine release (Driscoll *et al.*, 1994); that they cause greater impairment of alveolar macrophage clearance function (Oberdörster *et al.*, 1994); that they increase antioxidant levels in lung tissue (Janssen *et al.*, 1994); and that they lead to greater fibrotic reactions upon repeated exposures (Baggs *et al.*, 1992).

However, while our studies clearly demonstrated the higher pulmonary toxicity of ultrafine particles, such effects were not of an acute nature and did not include acute mortality. In fact, the exposure concentrations in our previous rat studies were rather high, in the 20 mg/m^3 range, which is far removed from environmental levels of less than $100 \text{ }\mu\text{g/m}^3$ associated with increased mortality in humans. On the other hand, there is a significant difference between environmentally-occurring ultrafine particles and those in our previous rat inhalation studies: Ambient ultrafine particles are inhaled as singlet particles whereas in our previous inhalation studies large aggregates of ultrafine particles were used. Generation of singlet ultrafine particles occurs during combustion processes, and our subsequent studies used thermodegradation of polytetrafluoroethylene (PTFE, Teflon[®]) to generate singlet ultrafine PTFE fume particles with the objective to determine their acute pulmonary toxicity in rats and to evaluate effects on work performance in rats after PTFE fume exposure. Initial results of our studies are presented here which demonstrate the extreme toxicity of low inhaled concentrations of singlet ultrafine fume particles leading to acute mortality in healthy rats.

METHODS:

A tube furnace (with 2 ft. stainless steel tube) which could be heated to temperatures of up to 1000°C was used as a PTFE fume generator. A small sample of PTFE (~400 mg) was heated in the center of the tube furnace at a controlled airflow of 5 l/min. Diluting air at a flow rate of 15 l/min. was added to the fumes after exiting the tube furnace and before entering the nose-only rat inhalation chamber. Particle concentration was measured by a combination of an electrostatic classifier (TSI model 371A) and a condensation particle counter (TSI model 3022A). In addition, particle samples were taken on a Nucleopore filter for SEM analysis and samples were collected for measurement of fluoride with a F-ion specific electrode (Orion, Model 96-09) to determine vapor phase fluor-compounds. Temperature in the nose-only exposure chamber in the breathing zone of the animals was about 4°C above ambient.

a. Acute toxicity studies:

Male Fischer-344 rats, bodyweight 200-230 g, were exposed to fumes generated at temperatures of 405, 420 and 425°C for 30 min. to evaluate acute pulmonary effects. Surviving rats were killed by an i.p. injected overdose of pentobarbital (100 mg) 4 hrs. after exposure and pulmonary lavage of the excised lung (10 x 5 ml saline) was performed. Cell differential, protein, LDH and β -glucuronidase were determined. Extreme toxicity of the PTFE fumes and mortality was noted in these initial studies, and since it was not our intention to determine the acute LC50 we performed additional range-finding studies in two animals per group only exposing them to PTFE fumes generated at 425°C at a particle concentration of $0.8 - 1 \times 10^6$ particles/cm³ for exposure durations ranging from 15-30 mins. Lung lavage parameters were determined 24 hrs. after exposure in the surviving animals.

b. Performance studies:

Twenty male Long-Evans rats maintained at a bodyweight of 270-290 g by food restriction were trained to run for one-hour sessions in a specifically-designed running wheel (Youssef *et al.*, 1993). (Based on our experience such training is more easily achieved with Long-

Evans rats rather than Fischer-344 rats.) For each 20 revolutions a reward of two 45 mg food pellets was given. Groups of 4 animals were exposed to PTFE fume concentrations of $0.74 - 1 \times 10^6$ particles/cm³ for a duration of 10 mins. (2 groups) or 20 mins. (1 group). The furnace temperature was kept at 425°C. Sham-exposed animals exposed for 20 mins. to heated filtered air served as controls. Performance was measured at 4, 24 and 48 hrs. after exposure and for up to 25 days post-exposure. Results were expressed as percent of the control values of each animal before exposure.

RESULTS:

Figure 2 shows the performance of the tube furnace with respect to generation of particle and fluoride concentration as a function of increasing furnace and PTFE temperature. Up to temperatures of about 405°C no significant amount of particles is generated, whereas between 415-425°C a steep increase in the amount of generated particles in the exposure chamber occurs reaching a level of about 1×10^6 particles/cm³ at a furnace temperature of 425°C. This steep increase in particle number is accompanied by an increase in fluoride up to a concentration of about 13 µg F/l. However, compared to the huge increase in particle numbers, amounting to several orders of magnitude, the fluoride increase is only modest, as depicted in Figure 2.

Determination of particle sizes with the electrostatic classifier confirmed that these PTFE fume particles indeed are in the ultrafine particle range as demonstrated in Figure 3. The median particle diameter is 26 nm with a geometric standard deviation of 1.4. A number concentration of 1×10^6 particles/cm³ of this size distribution is equivalent to a mass concentration of 64 µg/m³, assuming a particle density of 1.

A. Acute toxicity studies.

Results of our initial experiments demonstrated that increasing the heating temperature for generation of PTFE fumes over a narrow range from 405 to 425°C increases significantly pulmonary inflammatory responses as determined by the appearance of PMNs in pulmonary lavage 4 hours after exposure (Fig. 4): At 405°C heating temperature, no effects on

pulmonary lavage parameters were observed, whereas at 420°C exposure for 30 mins., 85% of the lavagable cells consisted of PMNs. At 425°C, none of the animals survived the 30 min. exposure to the 4 hr. timepoint, and the lavage fluid was bloody due to a large contamination with red blood cells indicating severe injury of the capillary-epithelial barrier. Histopathological evaluation of the lungs showed an acute hemorrhagic inflammatory edema. Lavage protein in the animals exposed to 420°C was also increased by a factor of 10 compared to control animals.

Figure 5 shows the results of the second set of experiments with animals exposed to fumes generated at 425°C for different exposure durations. Twenty-four hours after a 25-min. exposure, a significant increase in lavagable PMNs (6.4% of the lavagable cells, range 3.6 - 9.1%) could still be observed in the lung lavage fluid. Lavage protein levels were increased by a factor of 2 at this timepoint compared to controls. The two animals exposed for 30 mins. died shortly after the exposure as was seen in the previous experiment.

B. Performance studies.

Table 1 shows results of the effects of PTFE fume exposure on running wheel performance. Exposure for 10 min. to the PTFE fumes resulted in a significant decrease in work performance 4 hrs. after the exposure which persisted through 24 hrs. after exposure. In addition, even at this short exposure duration, 1 out of 4 animals in the group exposed to 7.4×10^5 particles/cm³ and 2 out of 4 animals exposed to a concentration of 1×10^6 particles/cm³ died. In the group exposed for 20 min. 3 out of the 4 animals died. One each of the surviving rats of Experiments 2 and 3 (Table 1) showed a persistent decrease in work performance over a 25-day post-exposure period (Fig. 6).

DISCUSSION:

We found that heating of PTFE to a critical temperature of ~415-420°C leads to the generation of fumes consisting of singlet ultrafine particles narrowly distributed around a median diameter of 26 nm. These fumes proved to be extremely toxic to rats, exhibiting a steep dose-response curve with respect to pulmonary inflammation and mortality within 10-15 min. of

exposure at particle concentrations in the range of $0.7\text{-}1.0 \times 10^6$ particles per cm^3 air. Conceivably, lower concentrations inhaled over a longer time period may also lead to adverse effects which needs to be examined. Acute mortalities were caused by hemorrhagic lung edema which, together with necrosis of the tracheobronchial epithelium and denudation of the alveolar epithelium, was also described in earlier studies by Lee *et al.* (1976). The calculated mass concentrations at which these effects occurred were on the order of $60 \mu\text{g}/\text{m}^3$, assuming unit density of the generated ultrafine particles. Physical performance of trained rats was also severely affected by exposures as short as 10 min. in duration, and even then resulted in the death of some animals.

Obviously, PTFE fumes consist of vapor phase constituents in addition to the ultrafine particle phase, and the question is whether the toxicity of the PTFE fumes is really due to the ultrafine particles or to vapor phase compounds or a combination of both. With respect to vapor phase compounds, Stavert *et al.* (1991) have shown that inhalation of hydrogen fluoride by rats in concentrations up to 1300 ppm results mainly in toxic effects in the upper respiratory tract whereas no significant changes were observed in the peripheral lung. In our experiments, fluoride concentrations approximated 10 ppm, far removed from the 1300 ppm used in the studies by Stavert *et al.* (1991). Other fluor-containing compounds in the vapor phase of PTFE fumes are carbonylfluoride and the highly toxic perfluoroisobutylene. Indeed, the high toxicity of PTFE fumes was initially attributed to fluorocarbon products such as carbonylfluoride which are generated when PTFE is heated above 490°C (Coleman *et al.*, 1968a, b). However, since carbonylfluoride was not found to be generated below PTFE temperatures of 490°C (Coleman *et al.*, 1968a) it cannot have played a role in our studies. With respect to perfluoroisobutylene, Lehnert *et al.* (1993b) found that exposure of rats to perfluoroisobutylene up to 10 ppm did not result in significant changes in the lung. Furthermore, Warheit *et al.* (1990) and Lee and Seidel (1991) have shown that removal of the particle phase by filters and exposure of rats to the vapor phase only did not lead to acute toxic effects as observed with the total PTFE fumes. All of these experiments speak strongly against a significant contribution of vapor phase constituents to

pulmonary toxicity and we believe that vapor phase fluoro-compounds are not significantly contributing to the acute toxicity and lethality of PTFE fumes generated at ~420°C.

Potentially, highly toxic radicals may be generated during the thermodegradation process and particles may act as carriers of these radicals to be deposited in and to affect the deep lung. Those radicals may be short-lived which might explain why aged PTFE fumes are not as toxic. Indeed, Seidel *et al.* (1991) could demonstrate that fumes generated from heating of several polymers contained toxic radicals. However, they also found that fumes from heating of other polymers did not contain such radicals and these were found to be as toxic as those showing radicals. Thus, it is not likely that short-lived radicals contribute significantly to the observed PTFE fume toxicity.

Finally, the well-known phenomenon that aged polymer fumes lose their toxicity (Lee and Seidel, 1991; Warheit *et al.*, 1990) could be explained by a process of particle aggregation with time which thereby diminishes their toxicity significantly. As indicated in the Introduction, our previous studies with aggregated ultrafine TiO₂ particles did show increased pulmonary toxicity compared to larger-sized TiO₂ particles, but the toxicity of these large aggregates of ultrafine particles was not nearly as high as that observed with the generated singlet ultrafine PTFE fume particles and did not cause acute or chronic mortality. We suggest, therefore, that inhaled singlet ultrafine particles of freshly-generated PTFE fumes are the likely cause of the high pulmonary toxicity and mortality observed in our studies. Obviously, particle concentrations of >10⁸ particles/cm³ are not very stable and will rapidly aggregate due to thermal motion and collision processes. However, particles at lower concentrations of 10⁶ and 10⁷ particles/cm³ are stable for several minutes before forming aggregates and losing toxicity.

In contrast to the very high number concentration, the mass concentration of such ultrafine particles is extremely low. If particle-induced pulmonary inflammatory processes are a function of the numbers of particles interacting both with inflammatory cells and with epithelial cells or a function of the particle surface area, ultrafine particles would be highly effective for inducing

adverse effects. For example, monodispersed particles of 20 nm diameter at a mass concentration of $10 \mu\text{g}/\text{m}^3$ would represent particle numbers of 2.4 million particles/ cm^3 , whereas the same mass concentration of a $2.5 \mu\text{m}$ particle (*i.e.*, particles in the fine size range, PM 2.5) would represent only a concentration of about 1 particle/ cm^3 (Table 2). We conclude that the extremely high numbers of singlet ultrafine particles play a significant role in the acute toxicity and mortality which occurred in our PTFE fume exposure studies in rats. The resulting lung injury also severely affects physical performance. We suggest further, as detailed in the following section, that ultrafine particles are at least partly responsible for acute human adverse effects observed in recent epidemiological studies (*e.g.*, Dockery *et al.*, 1993).

CONCLUDING REMARKS:

As mentioned in the Introduction, ultrafine particles have been measured in the environment (Fig. 1), and although they are not as persistent as larger particles they are constantly generated and replenished from combustion sources and by atmospheric reactions as condensation nuclei. Newer measurements by Castellani (1993) of an urban aerosol have shown that the number concentration of ambient ultrafine particles reaches well into the range of 10^5 particles/ cm^3 . He measured, in Bologna, Italy, the ultrafine particle phase in three different seasons of the year and found that ultrafine particle diameters ranged from 17 - 50 nm at concentrations up to 2.4×10^5 particles/ cm^3 . Figure 7 shows these results of ambient ultrafine particle concentrations in comparison to the concentrations used in our rat inhalation studies. Unless healthy rats are much more sensitive than humans with a compromised respiratory system, it is conceivable that these ultrafine urban aerosol concentrations can indeed lead to serious respiratory effects. Of course, ultrafine particles in the urban atmosphere are not PTFE particles and their nature still has to be determined, *e.g.*, whether they consist of soluble or highly insoluble materials, but the possibility that ultrafine particles have the potential to cause acute lung injury in humans should not be discounted. Reports about acute pulmonary edema of humans exposed to polymer fume particles (Rosenstock and Cullen, 1986; Makulova, 1965) show that humans are sensitive to the fume particle effects as well.

A significant fraction of the inhaled ultrafine particles has to deposit in the alveolar region of the lung in order to cause these local acute effects. The most recent human lung deposition model for different particle sizes proposed by the International Commission on Radiological Protection (ICRP, 1994) shows that ultrafine particles of ~20 nm in diameter have a 50% deposition efficiency in the alveolar region (Fig. 8). This is considerably higher than deposition of these particles in any other region of the respiratory tract including the nose, and is also considerably higher than deposition for any other particle size in the alveolar region. Such high deposition predisposes the ultrafine particles for effects in the peripheral lung. Moreover, a recent study by Anderson *et al.* (1990) showed that subjects with a compromised respiratory system, *i.e.*, obstructive lung disease, even have an increased deposition of inhaled ultrafine particles in their respiratory tract. Additionally, a pre-existing pulmonary disease means a weakening of the lungs' defenses which would lower further the tolerance for adverse effects of ultrafine particles. It is easily conceivable that a compromised lung will adversely respond to even lower concentrations of ultrafine particles than those used in our rat studies. Therefore, the effect of lower inhaled ultrafine particle concentrations needs to be determined, preferably in an animal model mimicking the diseased human lung. Another subset of the population, young children, may also react more sensitively to inhaled ultrafine particles; indeed, epidemiological studies demonstrating increased morbidity in children associated with particulate air pollution identified these as a sensitive group as well.

We conclude from our studies that inhaled singlet ultrafine particles of PTFE fumes are extremely toxic and cause acute mortality in rats at very low inhaled mass concentrations. Results from accidental exposures of humans to polymer fumes indicate that humans respond similarly. If such responses are generic to a wide variety of inhaled singlet ultrafine particles then those occurring in the urban atmosphere may particularly affect sensitive parts of the population at lower concentrations than used in the rat studies particularly if inhaled over a longer period of time. Thus, we need to determine whether the association between increased mortality/morbidity and low

level particulate air pollution can also be demonstrated for ambient ultrafine particles. This would call for a thorough characterization of the ultrafine urban aerosol fraction with respect to its physicochemical composition, concentration, persistence, and changes with specific atmospheric conditions. Finally, future research needs to identify the mechanisms of ultrafine particle toxicity with the aim to develop therapeutic measures.

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TABLE 1

RAT PTFE EXPOSURE: WORK PERFORMANCE (Mean \pm S.D.)

Exp. #	Exp. Duration (mins)	Part. Conc. $\times 10^{-5}$	Mort.	Performance		
				As Percent of Control 4 hr	24 hr	48 hr
1	Control	0	0/4	95 \pm 14	104 \pm 11	107 \pm 12
2	10	7.4 \pm 0.6	1/4 ^a	62 \pm 38	78 \pm 12	90 \pm 13
3	10	10.0 \pm 0.3	2/4 ^b	39 \pm 44	59 \pm 51	91 \pm 2
4	20	8.7 \pm 0.5	3/4 ^c	16 \pm 31	21 \pm 40	49 \pm 69

a = One rat died one day after exposure; b = two rats died one day after exposure;
c = one rat each died on day 1, 2 and 3 after exposure.

TABLE 2

NUMBERS AND SURFACE AREAS OF MONODISPERSE
PARTICLES OF UNIT DENSITY OF DIFFERENT SIZES
AT A MASS CONCENTRATION OF 10 $\mu\text{g}/\text{m}^3$

Particle Diameter μm	Particle Number per cm^3 air	Particle Surface Area, μm^2 per cm^3 air
0.02	2,400,000	3016
0.1	19,100	600
0.5	153	120
1.0	19	60
2.5	1.2	24

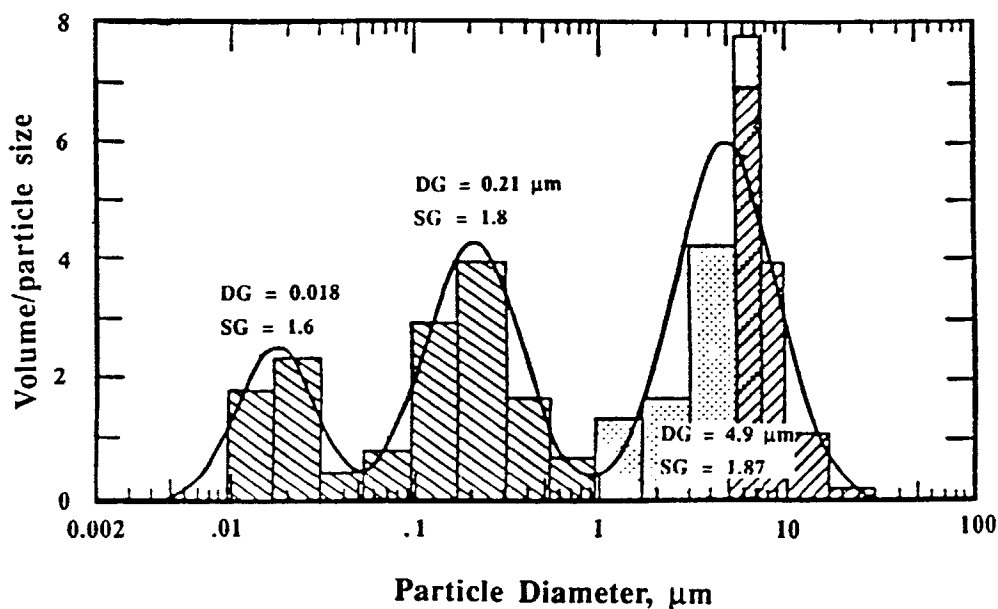


Fig. 1: Particle volume distribution of ambient aerosol measured by Wilson *et al.* (1977) in road tests. A typical trimodal distribution showing a coarse, an accumulation (fine), and a nuclei (ultrafine) mode is shown with geometric mean diameter of 4.9 and 0.21 μm and 18 nm, respectively. (SG=geometric standard deviation). (Adapted from Wilson *et al.*, 1977).

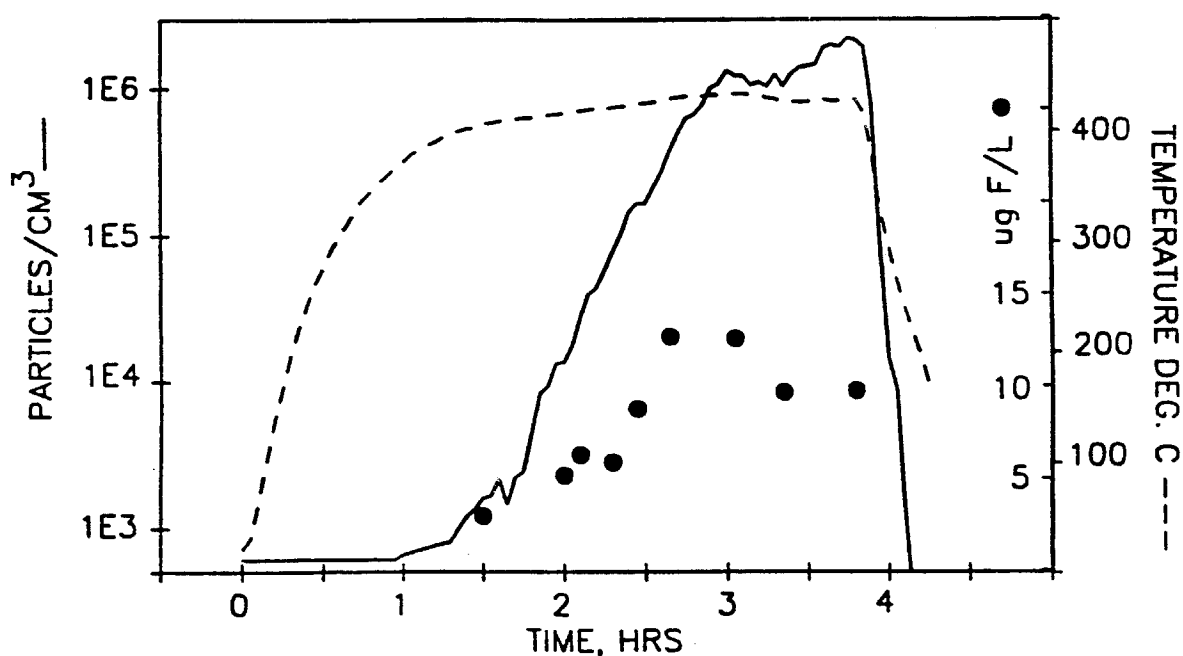


Fig. 2. Heating of PTFE above critical temperature leads to steep increase in ultrafine particle number concentration of PTFE fumes (note logarithmic scale) accompanied by lower increase in fluoride (linear scale).

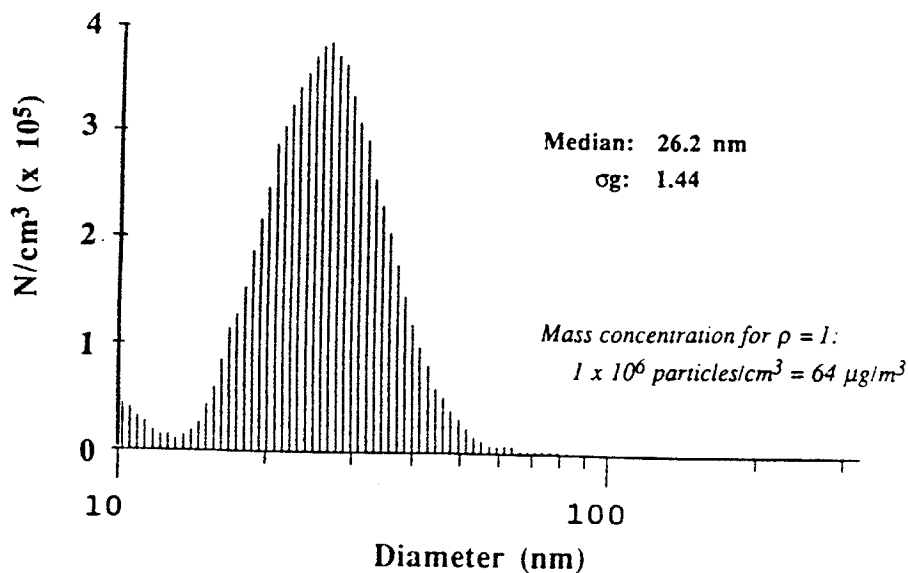


Fig. 3. Ultrafine particle size distribution of PTFE fumes generated at 420°C. Number median diameter is 26.2 nm with a geometric standard deviation of 1.44. Assuming particles of unit density a number concentration of 1×10^6 particles/cm³ would be equivalent to a mass concentration of 64 $\mu\text{g}/\text{m}^3$.

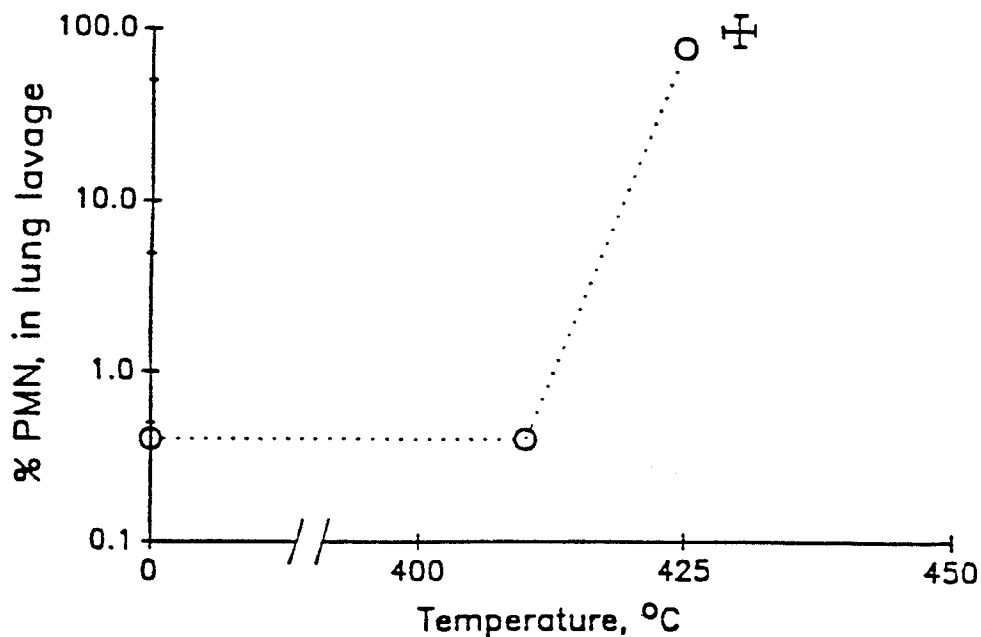


Fig. 4: PMN's in bronchoalveolar lavage fluid of rats 4 hrs after exposure for 30 mins. to PTFE fumes generated at different temperatures. $N = 2$ to 3 animals per data point. \oplus = all animals died during or shortly after exposure of severe hemorrhagic pulmonary edema.

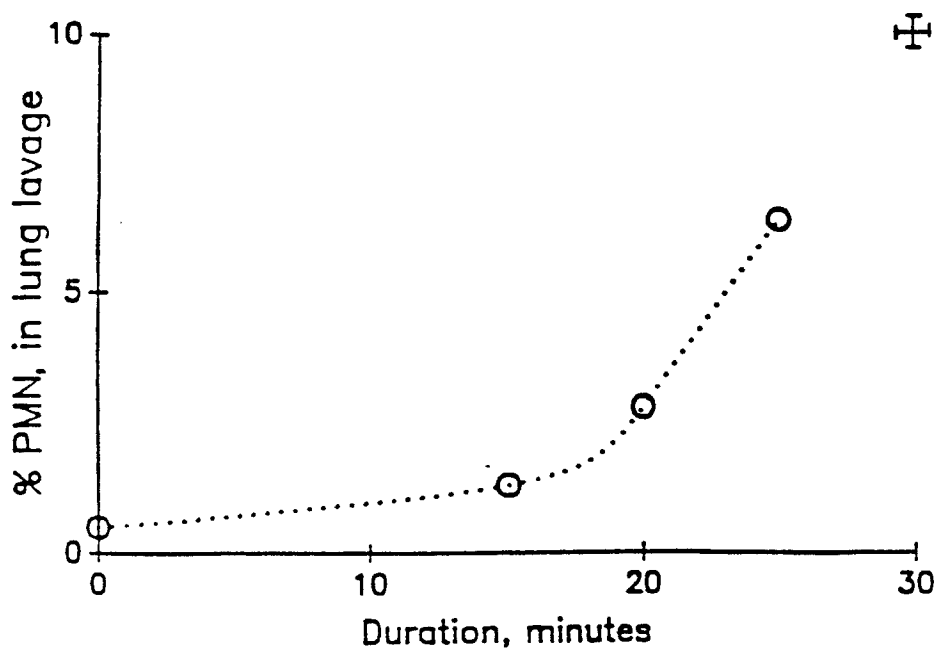


Fig. 5: PMNs in bronchoalveolar lavage fluid of rats 24 hrs. after different length of exposure to PTFE fumes generated at 425°C. N = 2 per data point. \oplus = all animals died during or shortly after exposure.

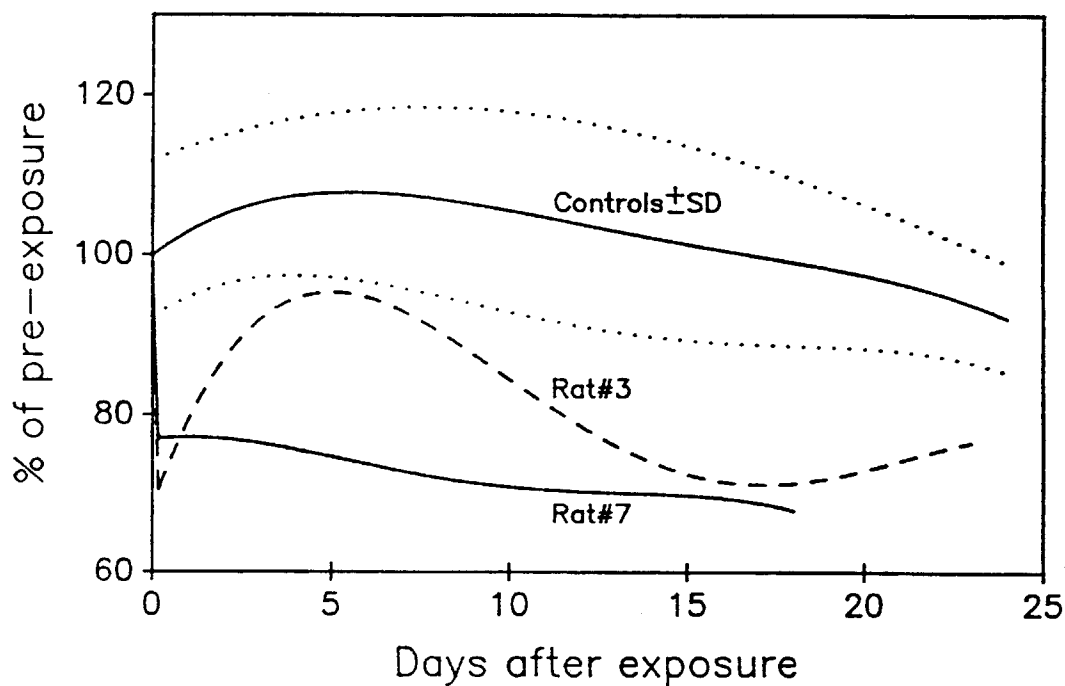


Fig. 6: Running wheel performance of rats after PTFE exposure as percent of pre-exposure level. Rats #3 and #7 were exposed for 10 mins. (Table 1). The control group represents the mean of a group of 4 sham-exposed rats.

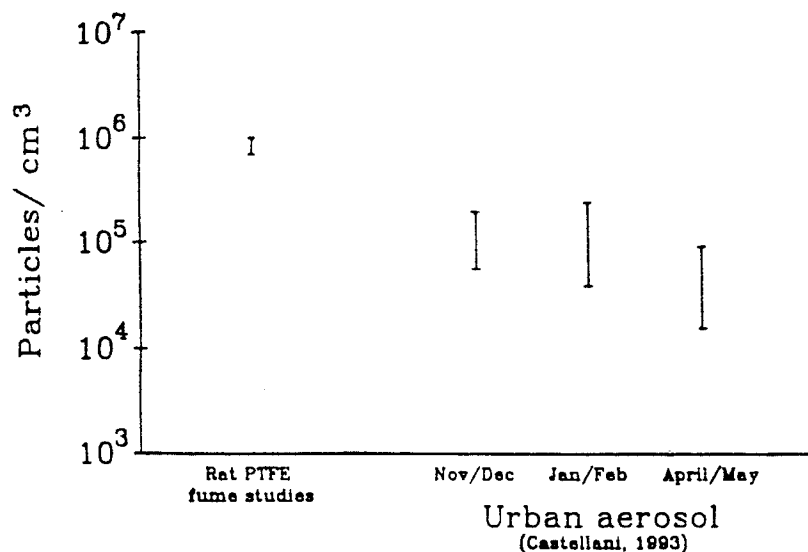


Fig. 7: Comparison of range of particle number concentration of ultrafine particles in urban aerosol of Bologna, Italy (Castellani, 1993) and in our PTFE fume rat inhalation studies.

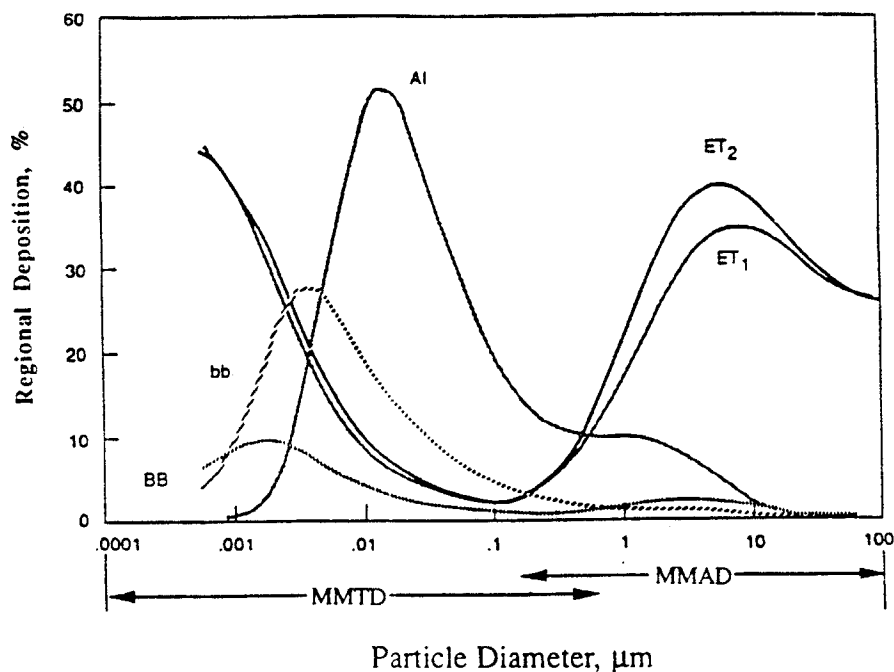


Fig. 8: Deposition efficiencies of inhaled particles in human respiratory tract during nose-breathing. Note the high deposition for ultrafine particles of ~20 nm diameter in the AI region of the lung. AI = Alveolar-Interstitial; BB = Bronchi; bb = Bronchioli; ET₁ = Anterior Nasal; ET₂ = Posterior Nasal, Pharynx Larynx; MMAD = mass median aerodynamic diameter; MMTD = mass median thermodynamic diameter. (Adapted from ICRP, 1994).

NUMBER CONCENTRATION AND MASS CONCENTRATION AS
DETERMINANTS OF BIOLOGICAL RESPONSE TO INHALED IRRITANT
PARTICLES ¹

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Abstract

Particulate pollutants are mixtures of a variety of chemical species. Sulfuric acid aerosol is a highly irritating component of particulate matter less than $10\mu\text{m}$ (PM₁₀) that can produce adverse health effects at current peak ambient concentration in the U.S.. We hypothesized that, in addition to the mass concentration of sulfuric acid, the number of sulfuric acid droplets was also an important factor affecting lung injury. To test this hypothesis, guinea pigs were exposed for 3 hr to either: filtered air; inert carbon particles at 10^8 particles/mL; sulfuric acid at $350\text{ }\mu\text{g SO}_4^{2-}/\text{m}^3$ layered on 10^8 , 10^7 , 10^6 /mL carbon particles; sulfuric acid at 50, 100, 200, and 300 $\mu\text{g SO}_4^{2-}/\text{m}^3$ layered on 10^8 /mL carbon particles. Alterations in phagocytic capacity (PC), intracellular pH (pHi), and intracellular free calcium concentration ($[\text{Ca}^{2+}]_i$) of harvested macrophages were used as indices of irritant potency. At a fixed number concentration of particles (10^8 particles/mL), there was a sulfuric acid concentration-dependent decrease in PC, pHi, and $[\text{Ca}^{2+}]_i$. Furthermore, at a fixed mass concentration ($350\text{ }\mu\text{g SO}_4/\text{m}^3$), sulfuric acid layered carbon particles at 10^8 particles/mL but not at other number concentrations decreased pHi of macrophages. The number concentration of sulfuric acid layered carbon particles did not affect PC or $[\text{Ca}^{2+}]_i$. These results suggest that there is a threshold for both number concentration and mass concentration for the aerosols to produce a biological response, and that epidemiologic studies should consider other aerosol characteristics in addition to mass when attempting to relate health endpoints to ambient pollutant exposures.

Introduction

Sulfuric acid (H_2SO_4) is an important component of the submicrometer particle mode of the atmosphere. It may exist in two forms: dissolved in aqueous droplets, and as a surface layer on solid particles. The first type of aerosol has been shown to produce alterations in a number of aspects of pulmonary physiology, biochemistry, and structure, and which seem to be proportional to both exposure concentration and exposure duration (U.S. EPA, 1989). The second type of aerosol is formed by adsorption of H_2SO_4 onto particles with sufficient surface area, such as typical ambient carbonaceous, metal, or fly ash particles. These particles are very potent in altering various markers of exposure. For example, reductions in lung volumes and diffusion capacity, alterations in biochemical markers, and changes in lung tissue morphology have been reported in guinea pigs after exposure to ultrafine zinc oxide having a surface layer of sulfuric acid (Amdur and Chen, 1989). The induced respiratory dysfunction is much greater than that due to H_2SO_4 aerosols in pure droplet form having similar size and concentration. A possible reason for this differential response is that the number concentration of particles in the exposure atmospheres are different, resulting in different numbers of deposition sites and local foci of reduced pH. At an equal sulfate concentration, sulfuric acid exists on many more particles when it is layered than when it is dissolved in aqueous droplets. Therefore, it is possible that the greater the number of particles containing sulfuric acid (as is the case with layered particles), the greater will be the number of cells affected after these particles deposit in the lung, and the more severe will be the overall biological response.

Since indirect evidence suggests that both total mass concentration of sulfuric acid as well as the total number of acid particles in the exposure

atmosphere may be critical parameters in determining the biological response, it was necessary to examine, in detail, the relationship between adverse health effects and these two exposure parameters. This paper describes a system to produce a controlled aerosol of carbon having a thin layer of sulfuric acid on its particles' surfaces. These aerosols were used to investigate the roles of mass concentration and number concentration in the response to acid sulfates. When using this system, we can produce sulfuric acid aerosols with varying total sulfur mass concentration at a fixed particle size and number concentration, or with varying number concentration at a fixed total sulfur concentration and fixed size. Moreover, the generated aerosols simulate those found in ambient air, where sulfuric acid may be layered on cores of carbon particles. The carbon particles generated in this study are toxicologically important for a number of reasons. First, because of their submicrometer size (approximately 0.05 to 0.1 μm), they are difficult to remove from combustion effluents and, once emitted into the atmosphere, they have a long residence time. Second, these particles penetrate deep into the lung. Third, they are commonly enriched in trace metals, which react with SO_2 to form a layer of H_2SO_4 on their surface. Fourth, the coarse, spiculated surface of the particles, as visualized by electron microscopy, enlarges the surface area for acid retention.

Experimental Design

Aerosol Generation System

Carbon aerosol having a thin layer of sulfuric acid on its surface was used in this study. The generation and characterization system is described in detail elsewhere (Chen, et al, 1994). Briefly, a high-temperature silicon carbide furnace was used to thermally decompose acetylene in argon to produce ultrafine carbon particles. The carbon particles were then diluted

and mixed with sulfuric acid droplets (produced by a Collison nebulizer) in a quartz tube maintained at 500°C to form a carbon aerosol with a thin layer of sulfuric acid. The particle number concentration was controlled by varying the dilution ratio of the first ejector dilutor. The amount of sulfuric acid on the particles was controlled by the bypass flowrate of the nebulizer. Aerosols were collected on membrane filters (Millipore Type FG, 0.2 µm pore size) and the sulfate concentrations determined using ion chromatography (Dionex Model 4000i). The number concentration was determined using a condensation particle counter (TSI Model 3020).

Animals

Male Hartley guinea pigs (virus antibody free) weighing 275 to 300g (Charles River Breeding Laboratories, Kingston, N.Y.) were housed 2 per cage in a temperature and humidity controlled room (23°C, 30-50% relative humidity) and allowed free access to food and water.

Animal exposures.

A Canon 52-port nose only exposure apparatus (Lab Products Inc.) was employed in this study to prevent neutralization of sulfuric acid by ammonia, which can occur in whole body exposures. The effect of number concentration on pulmonary response was investigated by exposing guinea pigs (n=6 per group) to sulfuric acid layered carbon particles at 10^8 , 10^7 , 10^6 particles/mL (total sulfuric acid mass concentration was maintained at $350 \mu\text{g SO}_4^{2-}/\text{m}^3$) for 3 hr. To investigate the effect of sulfuric acid mass concentration on pulmonary response, the animals were exposed to sulfuric acid layered carbon particles at 50, 100, 200, and $300 \mu\text{g}/\text{m}^3$ (number concentration for all these exposure was fixed at 10^8 particles/mL) for 3 hr. Separate groups of animals were exposed to filtered air or carbon particles alone (10^8 particles/mL) for comparison.

Bronchoalveolar lavage

Animals were anesthetized by injection (im) of ketamine hydrochloride (Ketaset, Bristol Lab. Syracuse, NY) and xylazine (Rompun, Haver Lockhart, Shawnee, KS), 100 mg/kg and 15 mg/kg, respectively, 24 hours after the end of the exposure. The animals were exsanguinated, the diaphragm was cut, and the lungs were lavaged *in situ* 4 times via a tracheal cannula with 7 mL aliquots of phosphate buffered saline (37°C). The first two lavages were combined and an aliquot was removed for total and differential cell counts and the remainder was immediately centrifuged (10 min at 400g). The supernatant was removed and assayed for total protein (BioRad, Richmond, CA) and lactate dehydrogenase (LDH) with commercially available kits (Sigma Chemical, St Louis, MO). Protein was assayed on frozen samples, while LDH was measured in lavage samples held at room temperature due to the sensitivity of this enzyme to freezing. Total cell counts were determined by manual counting on a hemocytometer. Cell viability was determined by the trypan blue exclusion technique. Differential counts were made on Giemsa-stained cells adhered to glass slides by cytocentrifugation. Data were expressed as the number of cells/mL and the number of each cell type recovered in the lavage.

The remaining two lavages were combined and centrifuged (10 min at 400g). The cell pellet was combined with the cells from the first 2 lavages and resuspended in RPMI 1640 (GIBCO, Grand Island, NY) and divided for the following measurements.

Assessment of phagocytic activity

The phagocytic activity of macrophages was evaluated using a suspension assay (Chen et al, 1992). Sterile polystyrene latex microspheres (3 μ m, Duke Scientific, Palo Alto, CA) were suspended in MEMH (GIBCO,

Grand Island, NY), supplemented with 10% heat-inactivated fetal bovine serum (FBS) and adjusted to 5.0×10^8 particles/mL. A volume of the original macrophage suspension was centrifuged (400g, 10 minutes), resuspended in RPMI 1640, and adjusted to 5×10^5 viable cells/mL. One-mL aliquots of the cell suspension were added to sterile polypropylene culture tubes and preincubated for 15 minutes at 37°C in a shaking water bath. The phagocytosis assay was initiated by adding 50 μ L of the opsonized latex particle suspension. Tubes containing the cells and particles were incubated for 60 minutes, after which time they were removed from the bath and cooled to 4°C. The particle-cell suspension was aspirated, smears prepared on glass slides by air-drying, and the cells fixed with phosphate-buffered, 2% glutaraldehyde (pH 7.2). The fixed smears were stained with Diff-Quik and placed in methylene chloride for 30 seconds to remove noningested particles. Three slides were prepared from each animal. On each slide, 100 macrophages were examined microscopically to determine the phagocytic capacity (PC). This measurement is an indication of the extent of phagocytosis, and was quantitated as the percentage of actively phagocytizing macrophages that had ingested 4 or more latex particles.

Measurement of Intracellular pH

Changes in intracellular pH (pHi) were monitored by use of the pH-sensitive dye probe, CARBOXY-SNARF-1, AM ACETATE (SNARF), according to the procedure described by Gillies and co-worker (1991). Briefly, the pulmonary macrophage (PM \emptyset) suspensions in HEPES buffer (135 mM NaCl, 5 mM KCl, 1 mM CaCl₂, 1 mM MgSO₄, 2 mM KH₂PO₄, 5 mM glucose and 10 mM HEPES) were loaded with 10 μ M SNARF at 37°C for 40 min. After incubation, extracellular SNARF was removed by washing the cells

three times with HEPES buffer. The cell pellets were finally resuspended in HEPES buffer at 5×10^6 cells/mL as the stock cell suspension for later use.

For the measurements of pHi levels of PMØ, aliquots of the cell suspension in HEPES (1.25×10^6 total cells) were centrifuged at 740g for 2 min, resuspended in 3.5 mL of HEPES buffer in a cuvette, and held in the fluorometer at 37°C with gentle stirring throughout the experiment. Fluorescence intensities were recorded on a Spex Fluorolog (Edison, New Jersey) with an excitation wavelength setting of 534 nm and emission wavelengths of 630 nm and 592 nm with excitation and emission slit widths of 4.5 and 9.0 nm, respectively. The ratio of emitted intensities at the two different wavelengths (R630/592) was used to calculate pHi from a calibration curve.

The pHi was calibrated using the K⁺-nigericin technique (Thomas et al, 1979). Briefly, dye-loaded cells were resuspended in high potassium solution (10 mM NaCl, 130 mM KCl, 1 mM CaCl₂, 1 mM MgSO₄, 2 mM KH₂PO₄, 5 mM glucose and 10 mM HEPES) and the pH of the medium was adjusted between 6.5 and 7.5 with KOH or HCl. An aliquot of the ionophore nigericin (1 µg/mL) was added to equilibrate pHi to the pH of the medium (pHo). The calibration curve was constructed by plotting pHo vs the corresponding emission ratio (R630/592).

Measurement of Cytosolic Calcium

Changes in intracellular calcium ([Ca²⁺]_i) were monitored by using a long wavelength calcium fluorescent probe, Fluo-3/AM (Minta et al, 1989, Kao et al 1989). Briefly, PMØ were loaded with 5 µM Fluo-3/AM (at cell density of 5×10^6 cells/mL) in HEPES buffer at 37°C for 40 min. After incubation, extracellular dye was removed by washing the cells three times

with HEPES buffer. The cell pellets were finally resuspended in the same buffer at 5×10^6 cells/mL as stock cell suspension for later measurements.

For the measurements of $[Ca^{2+}]_i$ levels of macrophages in HEPES buffer, aliquots of the stock cell suspension (0.25 mL) were centrifuged at 740 g for 2 min, resuspended in 3.5 mL of the same buffer in a cuvette, and held in the fluorometer at 37°C with gentle stirring throughout the experiment. Fluorescence intensity (F) was first recorded for forty seconds with an emission wavelength setting of 525 nm and an excitation wavelength of 506 nm and excitation and emission slit widths of 4.5 and 9.0 nm, respectively. The maximum fluorescence intensity (F_{max}) was then measured following the addition of 40 μ M digitonin and the minimum fluorescence intensity (F_{min}) was recorded after addition of 6 mM EGTA.

The $[Ca^{2+}]_i$ levels were calculated according to the following equation:

$$[Ca^{2+}]_i = 400 \text{ nM} * (F - F_{min}) / (F_{max} - F)$$

All chemicals used in this study were certified reagent grade.

Standards used to calibrate the instruments were NBS traceable. Distilled, deionized water with a resistance of 18 megaohms was used throughout the experiments. The fluorescent probes, SNARF-AM and Fluo-3/AM, were obtained from Molecular Probes (Eugene, OR) and dissolved in DMSO. Nigericin was purchased from the same source and dissolved in methanol for pHi calibration.

Statistical Analysis

All data are expressed as the mean % (\pm SEM) of filter air control values. Statistical comparisons of PM \emptyset phagocytosis function and ion concentration data were made using a one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls post-hocs test to identify any significant differences ($p < 0.05$) among group means.

Results

While none of the exposure groups showed alterations in the biochemical parameters of lavage fluid, there were alterations in pH_i of PMØs after exposures to sulfuric acid layered carbon particles. Carbon particles alone (10^8 particles/mL) produced a decrease in pH_i (0.052 pH unit). At a fixed number concentration of coated particles (10^8 particles/mL), a sulfuric acid concentration dependent decrease in pH_i (for concentrations of acid above $100 \mu\text{g SO}_4^{2-}/\text{m}^3$) was observed. For every $100 \mu\text{g}/\text{m}^3$ increase in sulfate concentration (for concentrations of acid above $100 \mu\text{g SO}_4^{2-}/\text{m}^3$), pH_i was decreased by 0.05 unit ($p = 0.0006$). The results are shown in Figure 1A. When animals were exposed to a fixed sulfate concentration ($300 \mu\text{g}/\text{m}^3$) of sulfuric acid layered carbon particles, a threshold for number concentration ($10^7/\text{mL}$) below which no decrease in pH_i was found.

As shown in Figure 2, carbon particles alone produced a 30.7% decrease in PC of PMØs. However, sulfuric acid layered on the same number of carbon particles ($10^8/\text{mL}$) stimulated phagocytosis at low sulfate concentrations (63 and $100 \mu\text{g}/\text{m}^3$), while sulfuric acid had no effect at higher concentrations (200 and $300 \mu\text{g}/\text{m}^3$). Linear regression analysis showed that for every $100 \mu\text{g}/\text{m}^3$ increase in sulfate concentration, there was a 8.6% decrease in PC of PMØs ($p=0.0005$). In contrast, at a fixed sulfate concentration ($300 \mu\text{g}/\text{m}^3$), different number concentrations did not produce alterations in phagocytosis.

As shown in Figure 3, at a fixed number concentration ($10^8/\text{mL}$) of acid coated particles, there was no change in $[\text{Ca}^{2+}]_i$ after exposure to either carbon particles alone or sulfuric acid layered carbon particles at any sulfate concentrations used in this study. However, linear regression analysis showed that for every $100 \mu\text{g}/\text{m}^3$ increase in sulfate concentration, there was

a 9.6 nM $[Ca^{2+}]_i$ decrease in PMØs. At a fixed sulfate concentration, different number concentrations of sulfuric acid particles had no effect on $[Ca^{2+}]_i$ of PMØs.

Discussion

By using an evaporation/condensation process, we produced a carbon aerosol with varying total sulfur concentrations, while maintaining the same particle size and number concentration. Using the same generation system, we also produced an aerosol with a fixed total sulfur concentration and fixed particle size while varying the number concentration. Because the particle size remains the same regardless of the number or mass (sulfur) concentrations, and because this type of aerosol is minimally hygroscopic, we did not create problems associated with pure sulfuric acid droplets, i.e. differential growth. Furthermore, the sulfuric acid was layered on core carbon particles, and its characteristics resembled those found in the ambient air.

Alterations in macrophage phagocytic function were observed despite the lack of significant changes in biochemical parameters in the lavage fluid of animals exposed to sulfuric acid layered carbon particles. Because the regulation and control of many cellular events, such as phagocytosis, are proposed to be mediated by changes in intracellular ion concentrations such as H^+ and Ca^{2+} , we also measured pH_i and $[Ca^{2+}]_i$ of PMØ. It appeared that alterations in these ion concentrations after exposure to sulfuric acid aerosols depend not only on the mass concentration of sulfuric acid layered on carbon particles, but also on the number concentration of particles in the exposure atmosphere. Regression analysis showed that $[Ca^{2+}]_i$ decreased with increasing sulfate concentration (at a fixed number concentration) as well as number concentration of sulfuric acid layered carbon particles (at a fixed

sulfate concentration). In addition, pHi decreased with increasing sulfate concentration (at a fixed number concentration) while changes in pHi did not occur until the number concentration of sulfuric acid layered particles was above $10^8/\text{mL}$. Thus, there appears to be a threshold for both the mass concentration as well as the number concentration of sulfuric acid layered carbon particles below which no change in pHi or $[\text{Ca}^{2+}]_i$ was observed.

The observation that the number concentration is a determinant of pulmonary response confirms, partially, the "Irritation-Signaling" model proposed by Hattis et al (1987). They hypothesized that since particle number per unit mass concentration declines dramatically with increasing particle size, there will be few in number relative to their mass and, therefore can deliver relatively few localized signals per unit of mass than smaller particles. Indeed, as observed in this study, alterations in pHi did not occur until the number concentration was above $10^8/\text{mL}$, suggesting that number concentration is a determinant of pulmonary response. The "Irritation-Signaling" model also suggests that particles smaller than about $0.4\text{-}0.7\ \mu\text{m}$ may not carry sufficient acid to cause damage. However, since the median particle size of the sulfuric acid layered carbon particles used in this study was $0.07\ \mu\text{m}$, the results of this study showed that particles smaller than $0.4\text{-}0.7\ \mu\text{m}$ can induce alterations in PMØs.

The importance of number concentration in eliciting pulmonary response found in this study also confirms our earlier finding that particle size and, therefore, particle number, is critical in determining the ability of acid aerosols to alter macrophage function (Chen et al 1992). In that study, different responses of PMØs to two different sizes of pure sulfuric acid droplets ($0.3\ \mu\text{m}$ and $0.04\ \mu\text{m}$) were observed. Since the same mass concentration of sulfuric acid was used for both aerosol size, a 420 times

greater number of 0.04 μm sulfuric acid droplets reached the alveolar region of the lung than 0.3 μm particles. Consequently, as shown in calculations in our previous study (Chen et al 1992), each macrophage located in the lumen of the alveolar air spaces was bombarded by 10 acid droplets of 0.04 μm size during the course of a 3 hr exposure, while only one out of 50 macrophages would be hit by a single 0.3 μm acid droplet. Therefore, as number concentration increases, more particles and, thus, higher doses are delivered to the macrophage population.

Although we did not compare the response of sulfuric acid layered carbon particles to that of pure sulfuric acid droplets, our previous studies (Amdur and Chen, 1989, Amdur et al 1989) had shown that sulfuric acid layered on ZnO particles was more potent in producing adverse pulmonary effects than sulfuric acid presented as pure droplets. Reductions in lung volumes and diffusion capacity (DLco), alterations of biochemical markers, and changes in lung tissue morphology were observed in guinea pigs after a single 3 hour exposure to ultrafine ZnO with a surface layer of sulfuric acid at sulfate concentrations as low as 60 $\mu\text{g}/\text{m}^3$. The significance of these results is demonstrated by the observation that it requires as much as 10 times more of a pure sulfuric acid droplet aerosol (same particle size) to produce the same magnitude of effect.

While animal experiments and human observational epidemiologic studies both suggest that acidic atmospheric environments can and do adversely affect humans, currently established clinical methods have generally failed to quantitate consistent lung function changes from exposures at or near ambient acid exposure levels in normals and in asthmatics exposed in controlled laboratory settings (U.S. EPA 1989). It may be that consistent significant effects are not occurring in the groups tested. On the other hand, it is also possible that the techniques employed

were not sufficiently sensitive to detect acid aerosol effects that may have occurred. A major gap in our knowledge of the human health effects of acid aerosols is related to the type of aerosol used in such controlled laboratory settings. Almost all of the published studies in this field have used aqueous aerosols of H_2SO_4 which have been produced by a variety of nebulizers. While these particles are generally of respirable size (less than $10\ \mu\text{m}$), they do not resemble the complex particles which are emitted from combustion processes used in the production of energy and which are a particular problem in the Northeast U.S. today.

The freshly generated combustion particles used in this study have the advantage of being similar to the combustion particles that occur in ambient air (Amdur et al, 1986), in contrast to the less realistic aqueous suspensions of larger carbon black particles recently tested by Anderson and co-workers (1991). In their study, aerosols of $100\ \mu\text{g}/\text{m}^3$ sulfuric acid applied to aqueous suspensions of carbon black aerosol did not elicit significantly different responses in normal subjects than did sulfuric acid alone by measures of SRaw, spirometry, and methacholine responsiveness. Because of their size and surface characteristics, freshly generated carbon particles, formed by thermal decomposition, are likely to be much more potent than the aerosolized slurry of carbon black particles and much more relevant to ambient acid aerosol pollution. In contrast, sulfuric acid layered either on carbon particles, such as the one used in this study, or on metal oxide, such as those used in our previous study, resemble those produced in the primary furnace effluent (Amdur et al 1986). Because of their size and surface characteristics, freshly generated carbon particles formed by thermal decomposition are likely to be much more potent than the aerosolized slurry of carbon black particles and much more relevant to ambient acid aerosol pollution.

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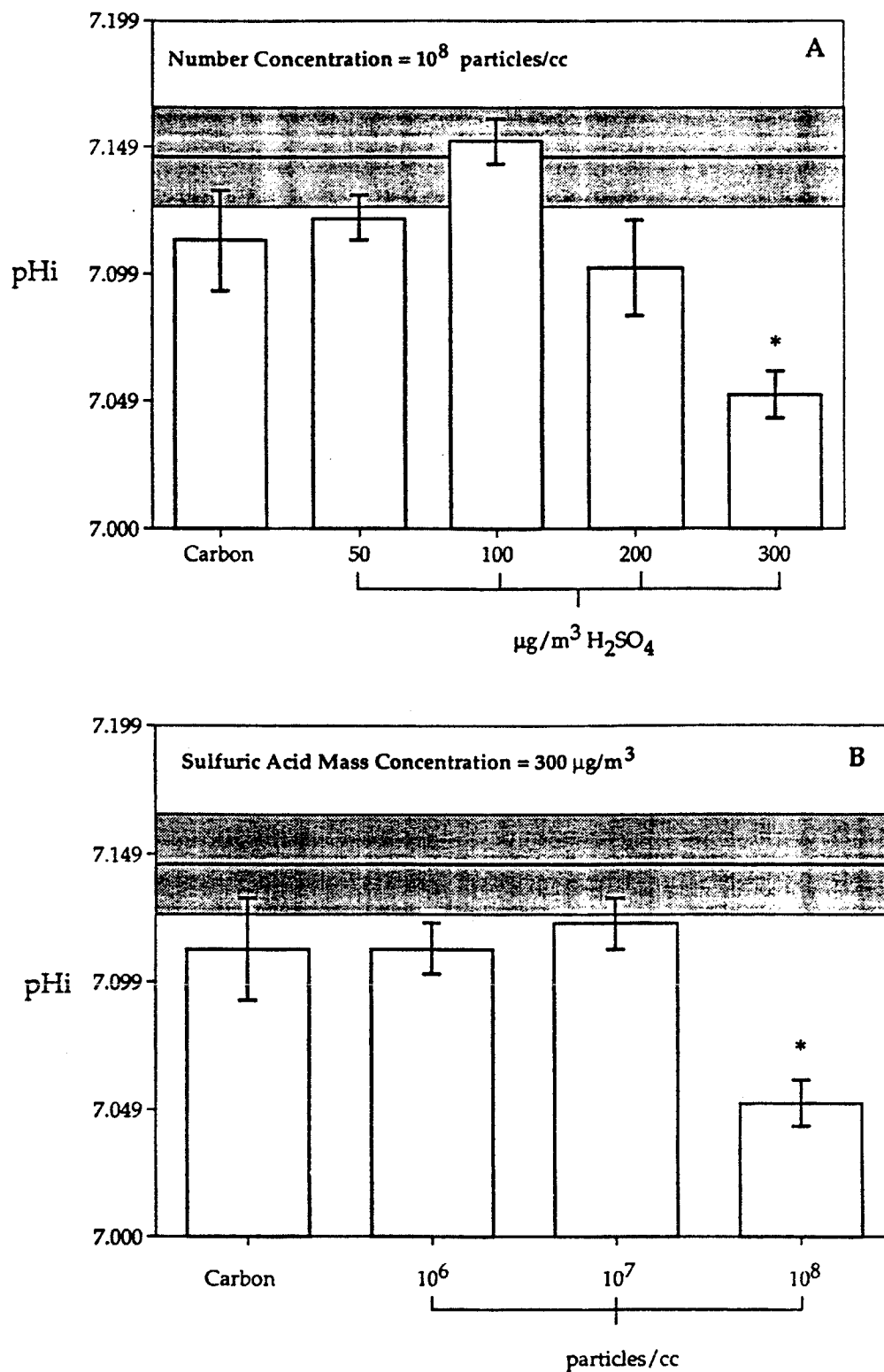


Figure 1. Effect of sulfuric acid layered carbon particles on pH_i of PMØs. A: Effect of total sulfate concentration at a fixed particle number concentration. B: Effect of particle number concentration at a fixed sulfate concentration. Shaded area indicates pH_i of control (mean ± S.E.). Values were mean ± S.E.. * indicates significant different from control (P<0.05).

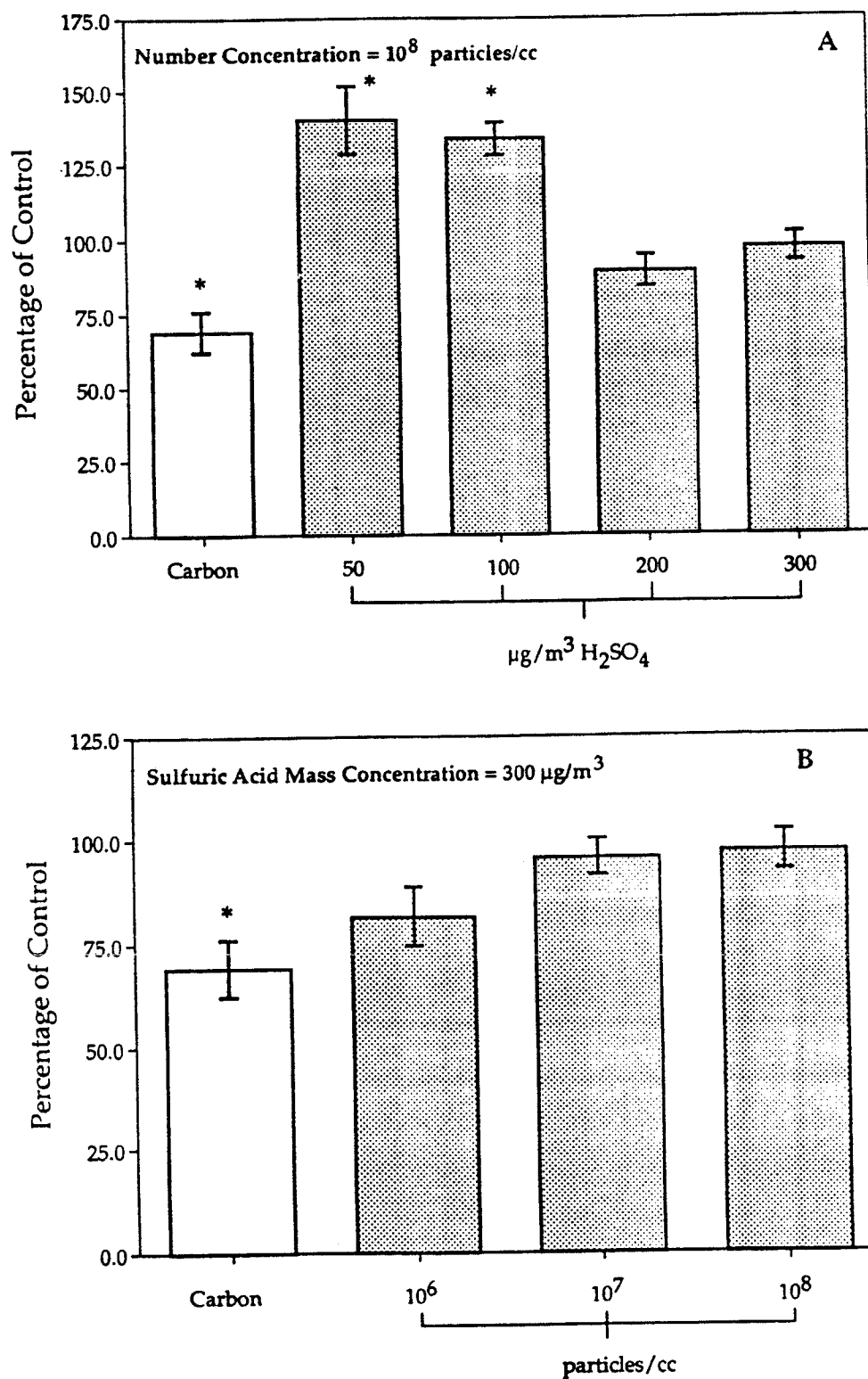


Figure 2. Effect of sulfuric acid layered carbon particles on phagocytic capacity of PMØs. A: Effect of total sulfate concentration at a fixed particle number concentration. B: Effect of particle number concentration at a fixed sulfate concentration. Values were percentage of control (mean \pm S.E.).

* indicates significant different from control ($P < 0.05$).

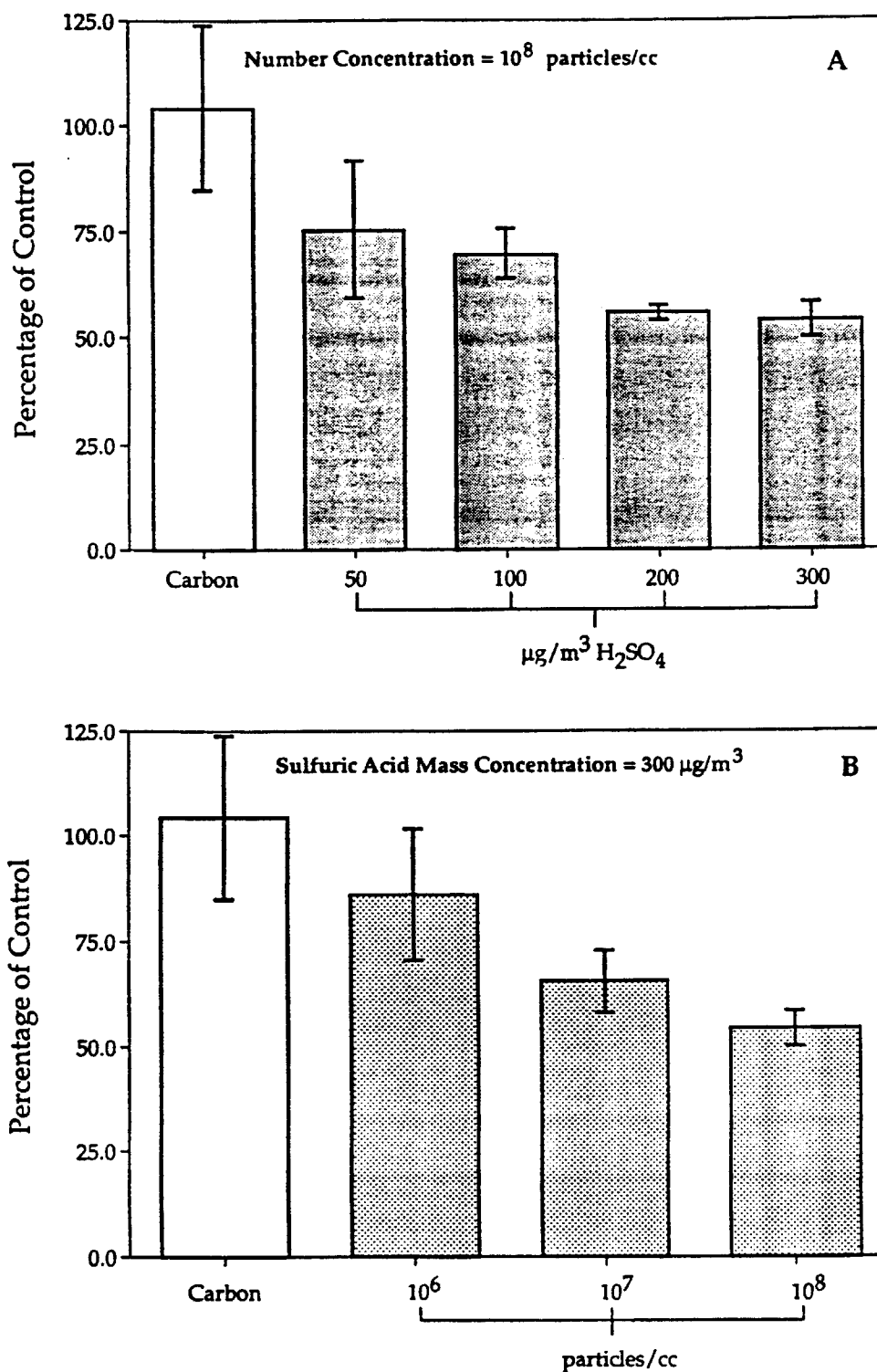


Figure 3. Effect of sulfuric acid layered carbon particles on $[\text{Ca}^{2+}]_i$ of PMØs. **A:** Effect of total sulfate concentration at a fixed particle number concentration. **B:** Effect of particle number concentration at a fixed sulfate concentration. Values were percentage of control (mean \pm S.E.).

* indicates significant different from control ($P < 0.05$).

**DEVELOPMENT AND EVALUATION OF A PROTOTYPE AMBIENT PARTICLE
CONCENTRATOR FOR INHALATION EXPOSURE STUDIES.**

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ABSTRACT.

A number of studies have underlined the importance of the acute and chronic effects of ambient particles on respiratory health. Because fine particles are capable of penetrating deeply into the respiratory system, most of the health studies have focused on the inhalable portion of the particle size spectrum. Previous studies to examine exposure/response relationships between particle exposure and adverse respiratory effects have been based on artificial preparations, or collected and resuspended ambient particles, rather than the natural material found in ambient air. Artificial particles may not be representative and collected particles may be difficult to redisperse. In addition, the chemical and physical characteristics of ambient particles may change upon resuspension.

We have developed a new technique that enables us to increase the concentration of ambient particles to levels about 10 times higher (or more, if desirable) than their ambient values and supply them to an exposure chamber. Ambient aerosol containing particles in the size range 0.1-2.5 μm can be concentrated using a series of two slit-nozzle virtual impactors. The intake flow rates are 1 m^3/minute and 200 liters/minute in the first and second virtual impactors, respectively. The virtual impactors were characterized in terms of their cutpoints and interstage losses using artificial monodisperse fluorescent aerosols as well as indoor ambient aerosols. Since the concentrated particles are

maintained airborne, they can be supplied to a human or animal exposure chamber for conduction of exposure studies. The supply flow rate in the prototype concentrator is 40 liters/minute. Higher flow rates can be achieved by using more than one such systems in parallel.

INTRODUCTION

A number of studies have underlined the importance of the acute and chronic effects of ambient particles on respiratory health. Ambient particles can consist of natural materials such as pollen, and anthropogenic materials such as acid aerosols. Also, particles can be directly emitted by different sources (primary aerosols), or be formed during the gas-to-particle conversion process (secondary aerosols). Both the concentration and the size of the particles depend on a number of factors such as the nature of the emissions, photochemical activity, and meteorological events. Since particle size is one of the most important parameters of aerosols, particles are usually classified into two categories: coarse (particle aerodynamic diameter $> 2.5 \mu\text{m}$) and fine (particle aerodynamic diameter $\leq 2.5 \mu\text{m}$). The first category generally includes particles produced through mechanical processes acting on such materials as soil or water, while the second category includes particles formed through gas-to-particle conversion as in the case of acid sulfates. Because fine particles are capable of penetrating deeply into the respiratory system, most of the health studies have focused on the

inhalable portion of the particle size spectrum. Twenty years ago, the U.S. EPA included Total Suspended Particles (TSP) as one of eight criteria pollutants. Recently, TSP has been replaced by PM_{10} (particles with an aerodynamic diameter of less than $10\ \mu m$), since PM_{10} is a measurement more relevant to respiratory health. Considering that the chemical composition and toxicity of particles can vary with time and location, particle mass measurements may not alone be a sufficient criteria for protecting the public health. For instance, exposure to an equal amount and particle size of soil dust and acid aerosol may not necessarily result in similar adverse respiratory health effects.

The literature on health effects of particulates published prior to 1981 has been reviewed by Ware et al. (1981). Particle levels have been shown to be significantly associated with respiratory and cardiovascular disease in numerous mortality studies (Fairly, 1990; Schwartz and Marcus, 1990; Pope et al., 1992). Field studies of adverse respiratory effects of particles conducted in a relatively high exposure community have shown significant associations between PM_{10} exposure and a number of morbidity outcomes, including hospital admissions for bronchitis and asthma, and longitudinal changes in peak flow rates, respiratory symptoms and medication use (Pope et al., 1991; Pope et al., 1992). Numerous laboratory studies have been reported in which healthy and asthmatic subjects were exposed to particle preparations. These studies have been performed almost exclusively with liquid acid aerosols (Avol et al., 1988; Koenig et al., 1988;

Hackney et al., 1989). Anderson et al., (1992) have exposed humans to carbon particles coated with sulfuric acid in order to simulate carbonaceous/acid aerosol particle mixtures. Modest or no adverse respiratory effects have been observed in these studies, even with the use of considerably higher exposure levels (up to 1500 $\mu\text{g}/\text{m}^3$ in Avol et al., 1988, and 2000 $\mu\text{g}/\text{m}^3$ in Hackney et al., 1989), than have been associated with equal or larger adverse effects in epidemiological studies (up to a maximum daily levels of 195-365 $\mu\text{g}/\text{m}^3$ of PM_{10} in Pope et al., 1991, Pope and Dockery, 1992). This discordance between laboratory and epidemiological studies may be explained by the relatively short duration of exposure in the laboratory studies. Alternatively, the difference in the findings may be indicating that the artificial particles do not replicate the adverse effects of the complex and heterogeneous mixtures that actually occur in ambient air.

Our rationale is to develop a new technique that enables us to increase ambient particle concentrations by a factor of approximately 10, or more, if desirable, to conduct controlled human exposure studies. Particle concentration is increased by separating ambient particles from gases in a virtual impactor. Phase separation occurs in few microseconds, thus the concentrated particles are in equilibrium with the same ambient gaseous pollutant concentrations. Furthermore, the concentration of gaseous pollutants (such as O_3 , NO_x , HCHO , HCOOH), temperature and relative humidity can be controlled; additional gas phase pollutants could be added, if desirable, or removed using diffusion

denuders. The results of this pilot study will be used for the construction of a larger concentrator system suitable for nose-only animal exposures to ambient aerosols at concentration levels up to few mg/m^3 .

METHODS

Design and Description of the Components

The concept of the virtual impactor is used for the construction of the aerosol concentrator for a desired particle range. The virtual impactor is a device used for the classification of particles according to their aerodynamic size (Figure IV.1). In this device, a jet of particle-laden air is injected at a collection probe, which is slightly larger than the acceleration nozzle (Loo et. al, 1988). Particles larger than a certain size cross the air streamlines and enter the collection probe, while particles smaller than a certain size follow the deflected streamlines. In order to remove the larger particles from the collection probe, a fraction of the total flow is allowed to pass through the probe, referred to as the minor flow (typically 10% of the total flow). As a result, the concentration of the larger particles in the minor flow has increased by a factor of Q_T/q_m , where Q_T is the total flow entering the virtual impactor and q_m is the minor flow. Since the mass fraction of ambient particles smaller than approximately $0.1 \mu\text{m}$ aerodynamic

diameter is negligible (Whitby et al., 1972; McMurry and Zhang, 1990), the minor flow of a virtual impactor with a 50% cutpoint on the order of 0.1 μm contains essentially most (90% or more) of the fine ambient particulate mass.

The ambient particle concentrator (Figure IV.2) consists of the following components:

- a. A high volume conventional impactor with a 2.5 μm cut-off size (separator).
- b. Two virtual impactors with a 0.15 μm cut-off size (stages I, and II).

The first impactor is a high volume conventional impactor (Fractionating sampler, Andersen Samplers) and removes particles larger than 2.5 μm operating at 1000 liters/minute, while particles smaller than 2.5 μm escape collection. The impactor has been characterized in detail elsewhere (Burton et al., 1973).

The deflected flow of the first impactor, containing particles smaller than 2.5 μm in aerodynamic diameter (1000 liters/minute), is drawn through a series of virtual impactors (Figure IV.2). Once all the particles larger than 2.5 μm have been removed, the concentration of particles smaller than this size could be increased by separating the particles from the gas in a virtual impactor of a cutpoint equal to 0.1 μm . In this case, the minor flow of the virtual impactor would contain the concentrated aerosol. Ideally, the cut-off size of the virtual impactor should be as small as possible. However, it is not trivial to design an

impactor with a very small cutoff size: high velocities and very low pressures are required. Koutrakis et. al (1989) demonstrated that the size of atmospheric sulfate particles varies from 0.2 to 1.0 μm with an average around 0.5 μm depending on the relative humidity. A study by Hitzenger and Puxbaum (1993) demonstrated that over 90% of the fine mass of urban aerosols was associated with particles larger than 0.10 μm in size. In addition, the distributions of particulate SO_4^{2-} , NO_3^- , and NH_4^+ were nearly identical with a mass median diameter about 0.7 μm . Thus, a reasonable compromise in terms of the exposure studies that will be conducted using this sampling system would be to design the virtual impactor at a 0.1 μm 50% collection efficiency size. With this method, an increase in the concentration of particles larger than 0.1 μm and smaller than 2.5 μm in aerodynamic diameter can be achieved.

If an increase in the concentration by a factor of 10 is desired, the virtual impactor has to operate at a minor-to-total flow of 10%. However, several studies have shown that interstage losses, a major area of concern with virtual impactors, tend to increase dramatically as the minor-to-total flow ratio decreases (Loo et al., 1988; Marple and Chien, 1980). For a minor-to-total flow ratio on the order of 10%, the particle interstage losses can be as high as 80% near the impactor's cutpoint. Particle losses can be drastically reduced by having the virtual impactor operate at a minor-to-total flow ratio of approximately 20%. In addition, the collection efficiency of the impactor is increased at a higher minor

flow ratio, since more particles penetrate the collection nozzle of the impactor, where the actual particle-gas separation occurs (Loo et al., 1988). By using two virtual impactors in series, each operating at a minor-to-total flow ratio of 0.2, the concentration of ambient aerosols can be increased to the desired level without considerable particle losses.

The first virtual impactor (Stage I), shown in Figure IV.1, has a slit-shaped acceleration nozzle which is 28 cm long and 0.03 cm wide, and a collection slit-shaped nozzle, 0.045 cm wide and 28 cm long. The ratio of the collection-to-acceleration slit widths was chosen equal to 1.5 to minimize particle losses typically occurring at the tip of the collection slit, as recommended by Loo et al. (1988). The impactor operates at 1000 liters/minute, with a minor-to-total flow equal to 0.2.

The minor flow of Stage I (200 liters/minute) is drawn through a second virtual impactor (Stage II), with the same particle cutpoint size. The virtual impactor consists of a slit-shaped acceleration nozzle, 0.03 cm wide and 5.6 cm long, and a slit-shaped collection nozzle, 0.045 cm wide and 5.6 cm long. The minor flow, equal to 40 liters/minute, contains the concentrated ambient aerosol consisting of particles in the size range 0.1-2.5 μm .

Experiments with monodisperse fluorescent aerosols

The second virtual impactor (stage II) has been evaluated in laboratory tests using fluorescent monodisperse aerosols with a method previously described by Raabe et al., (1988). Briefly, suspensions of 2.5% by weight yellow-green latex microspheres (Fluoresbrite, Polysciences, Warrington, PA) were nebulized by a pocket nebulizer (Retec X-70/N) using room air at 20 psi. The nebulized aerosol passed through a 1-liter chamber with ten Polonium 210 ionizing units (Staticmaster, NRD Inc.) to reduce the particle charge to Boltzmann equilibrium. After the neutralizer, the aerosol was mixed with room air in a 20 liter chamber and passed through the test system, which consisted of the virtual impactor, followed by two 47 mm glass fiber filters connected to the major and minor flows to collect the test particles. Each filter was connected to a pump with a rotameter in line to control the air flows. Another part of the test aerosol was drawn through an optical particle size analyzer (model LAS-X Particle Measuring System, Inc., Boulder, CO) to measure the particle size distribution throughout the experiment. In addition, a 4.7 cm glass fiber filter was connected in parallel to the test system to provide an estimate of the concentration of the generated fluorescent aerosol. Finally, the pressure drop across the virtual impactor was continuously monitored in every experiment with a Magnehelic pressure gage (range 0-100 inches H₂O). At the end of each run, each glass fiber filter was placed in 5 ml of ethyl acetate to extract the fluorescent dye from the collected

particles. The filters and the solution were ultrasonicated for a few minutes. The quantities of the fluorescent dye in the extraction solutions were measured by a fluorometer to determine particle concentration. The collection efficiency of the virtual impactor was determined by dividing the amount of fluorescence collected on the minor flow filter to the sum of the amounts collected on both major and minor flow filters. Furthermore, particle losses were determined by comparing the concentration determined with the control filter to the total concentration in the major and minor flows of the virtual impactor.

Tests with Ambient Aerosols

Indoor aerosol sampling experiments were conducted at the Harvard School of Public Health during the winter 1993-94, using room air as the test aerosol. The second virtual impactor (stage II) was characterized separately from the rest of the components of the concentrator. Subsequently, the entire concentrator was tested. The ambient levels of fine particle mass ($PM_{2.5}$) and particulate sulfate were estimated using two collocated Harvard-Marple Impactors (HMI). The Harvard-Marple Impactors (HMI) have been designed and characterized to have a 50% aerodynamic diameter cutpoint of $2.5\ \mu m$ at a flow rate of 4 liters/minute (Marple et al., 1987). The same study reported negligible interstage particle losses ($<0.2\%$) for particles smaller than $2.7\ \mu m$ in aerodynamic diameter. Sampling duration varied from 24 to 48 hours, depending

on the observed air quality levels.

Measurements were conducted by placing 47 mm Teflon filters downstream of the major and minor flows of Stage II and identical Teflon filters downstream of the Harvard-Marple impactors and comparing the fine particulate mass and sulfate concentrations ($d_p < 2.5 \mu\text{m}$) determined by both sampling methods. The ratio of the concentration in the minor flow of stage-II to the average concentration of the two HMI's gives an estimate of the ambient particle concentration levels that were achieved. The filters were preweighed on a Cahn 31 electrobalance in a room with constant temperature and humidity. After each run, the filters were weighed in the same electrobalance, after equilibration in the constant humidity and temperature room. After their final weighing, the filters were cut and placed inside a polycarbonate vial. The filters were extracted with 5 ml of ultrapure water and 0.100 ml ethanol, which wets the typically hydrophobic Teflon filters. Subsequently, the filters were sonicated for 15 minutes and analyzed for sulfate ions by ion chromatography (Koutrakis et al., 1988).

RESULTS AND DISCUSSION

The collection efficiencies and particle losses of Stage II are shown in Figure IV.3. The 50% cutpoint is $0.10 \mu\text{m}$, and the particle losses range from 5-

15%. Particle losses tend to slightly increase with particle size, probably due to inertial deposition of large particles on the tip of the narrow collection slit. The collection efficiency curve of the virtual impactor is not as steep as a typical curve of a conventional impactor (Marple et al., 1987). The non-ideal inertial behavior of particles as small as $0.1\ \mu\text{m}$ in size is one possible explanation. Particles in this size range have non-negligible diffusion, and there may be several other mechanisms that throw small particles into the collection probe (for example, the development of eddies around the acceleration nozzle, which has been shown by Masuda et al., 1988, and by Forney et al., 1982).

To investigate the effect of the minor-to-total flow ratio on the performance of the virtual impactor, the minor flow of stage II was varied from 5 to 40 liters/minute (or, equivalently, the minor flow ratio, q_m/Q_T , was varied from 0.025 to 0.20), while the total sampling flow was maintained constant at 200 liters/minute. Indoor air were used as the test aerosol. Results of this investigation are shown in Table IV.1. The collection efficiency was evaluated by dividing the amount collected in the minor flow to the sum of the amounts in the minor and major flows.

Table IV.1. Characterization of stage-II virtual impactor using ambient aerosols.

Minor Flow Ratio (%)	Efficiency (%) ^a	Particle Losses (%) ^b	Concentration Factor ^c
20	80.3	10.4	3.6
20	79.8	15.3	3.6
20	81.3	11.2	3.6
10	64.2	37.8	3.9
10	59.2	26.1	4.4
7	50.6	38.8	4.3
2.5	49.8	50.3	10.0
2.5	52.3	59.2	8.6

a. Efficiency is defined as the amount collected in the minor flow divided by the sum of the amounts collected in the minor and major flows of the virtual impactor.

b. Particle losses (PL) are defined as $PL = 1 - ((m + M)/Q_T) / (H/Q_{HMI})$, where *m* and *M* are the amounts collected on the filters of the minor and major flows, respectively, Q_T is the total flow of stage II (200 LPM), *H* is the amount collected on the filter of the Harvard-Marple Impactor, and Q_{HMI} is the flow rate of the

Harvard-Marple Impactor (4 LPM)

- c. Concentration factor is the ratio of the concentration in the minor flow to the ambient $PM_{2.5}$ concentration estimated by the Harvard-Marple Impactors.

The collection efficiency decreases and particle losses increase dramatically as the minor-to-total flow ratio decreases. This is due to the fact that a higher minor flow results in a higher local velocity around the tip of the collection nozzle, and consequently a stronger vacuum is applied to the particles as they exit the acceleration nozzle and approach the proximity of the collection probe. A stronger force therefore pulls more particles into the collection probe, resulting in lower deposition around the tip of the collection nozzle. These results are in agreement with previous experimental investigations that attempted to optimize the design of virtual impactors (Loo et al, 1988; Sioutas et al., 1994). Although the overall increase in the concentration becomes higher at lower minor flow ratios, the performance of the impactor is deteriorated due to the increased losses and decreased collection efficiency, both of which can distort the size distribution of the sampled ambient aerosol. If an increase in the ambient aerosol concentration by a factor of 10 is desired, two stages operating at a minor flow ratio of 0.20 each are recommended over one stage operating at a minor flow ratio of 0.025, since the former configuration achieves the same concentration increase with high efficiency and low losses per stage, thus minimizing distortion of the initial size distribution of the sampled aerosol. In addition, for the same

total sampling flow rate, the two-stage, 0.20 minor flow ratio configuration provides concentrated aerosols at a higher flow rate to the exposure chamber than the single-stage configuration.

The results of the prototype concentrator evaluation are shown in Table IV.2. In all the experiments, the relative humidity was recorded and found to be 30% or lower. The ambient fine mass ($PM_{2.5}$) mass concentration ranged from approximately 10 to 20 $\mu\text{g}/\text{m}^3$, whereas the particulate sulfate concentration varied from approximately 2 to 4.5 $\mu\text{g}/\text{m}^3$. The experimental results indicate that the increases in the concentrations of ambient fine mass and ambient sulfates achieved in the concentrator agree well with each other in every experimental run. The ambient fine mass concentration is increased by an average factor of 8.9 (± 0.3), whereas the ambient sulfate concentration is increased by a factor of 8.2 (± 0.3). The agreement in the concentration increase between fine mass and sulfates implies that the size distributions of sulfates and fine particles may be similar.

Table IV.2. Characterization of the concentrator using ambient indoor particles as the test aerosol.

Exp. No.	Ambient Fine Mass ($\mu\text{g}/\text{m}^3$)	Concentrator Fine Mass ($\mu\text{g}/\text{m}^3$)	Ambient Sulfate Concentration ($\mu\text{g}/\text{m}^3$)	Concentrator Sulfate Concentration ($\mu\text{g}/\text{m}^3$)
1	17.3	122.3	4.0	24.6
2	13.0	122.9	3.3	28.1
3	11.3	114.1	3.4	27.8
4	11.6	102.6	2.6	21.1
5	18.1	151.3	3.0	28.2
6	20.8	186.4	4.3	30.2
7	22.1	200.6	4.8	40.7
8	8.9	81.9	2.2	20.4
9	10.9	115.5	2.5	22.8
10	15.1	135.4	2.9	25.1

CONCLUSIONS

A prototype aerosol concentrator using the principle of virtual impaction has been designed and evaluated. Ambient aerosols are concentrated as they pass through two virtual impactors in series, each one containing the majority of ambient fine mass ($d_p < 2.5 \mu\text{m}$ aerodynamic diameter) in a bleed flow that is 20% of the total flow entering the virtual impactor. In each stage, the concentration is increased by a factor of 3-3.5.

The concentrator has been evaluated using monodisperse fluorescent aerosols, as well as indoor air particles. The experimental results indicated that the two-stage concentrator increased the concentration of ambient fine particles by a factor of 8.9 (± 0.3), and the concentration of ambient sulfates by a factor of 8.2 (± 0.3). All of the experiments were conducted at relative humidities lower than 30%, and since the average sulfate size increases at higher relative humidities, the achieved concentration factor values may represent conservative estimates.

The developed system is suitable for human exposure studies, since it can supply concentrated aerosols to an exposure chamber at high flow rates (40 LPM). Higher supply flow rates can be achieved by operating more than one system in parallel. The addition of one or more stages in series can raise ambient

fine particle concentrations even further. We are currently developing a unit that utilizes three stages to increase fine particle concentrations 30-40 times their ambient values. This system will be used for animal exposures, in which higher concentrations are desired.

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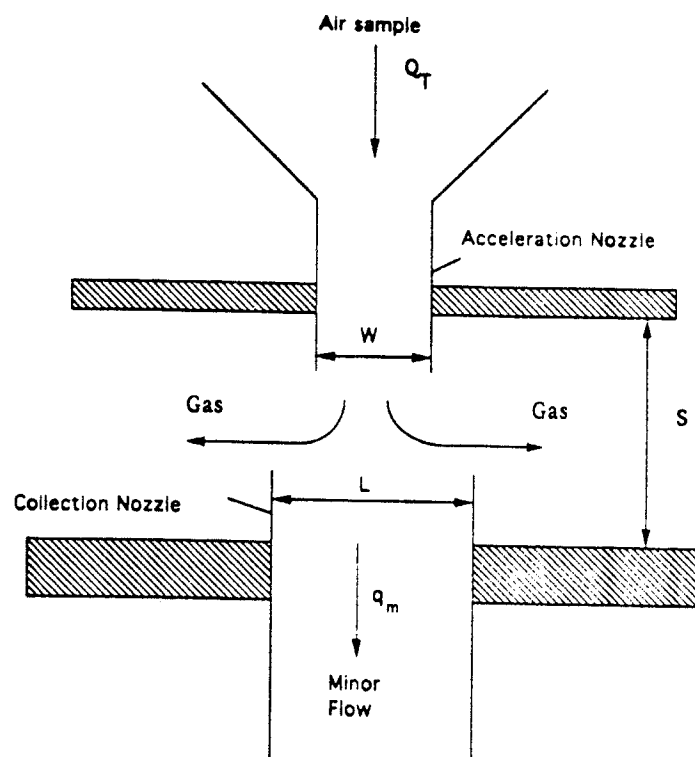
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Nozzle detail

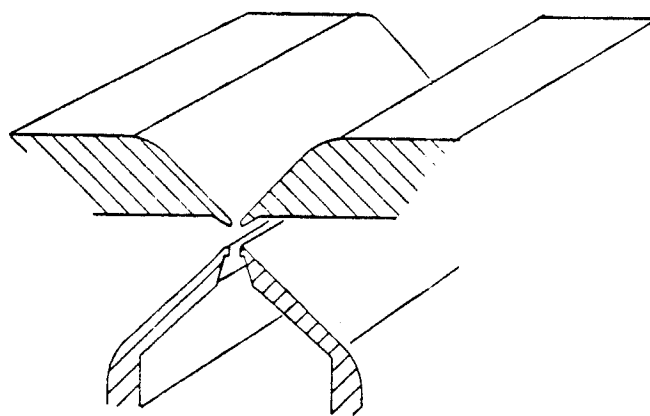


FIGURE 1. Schematic diagram of a virtual impactor.

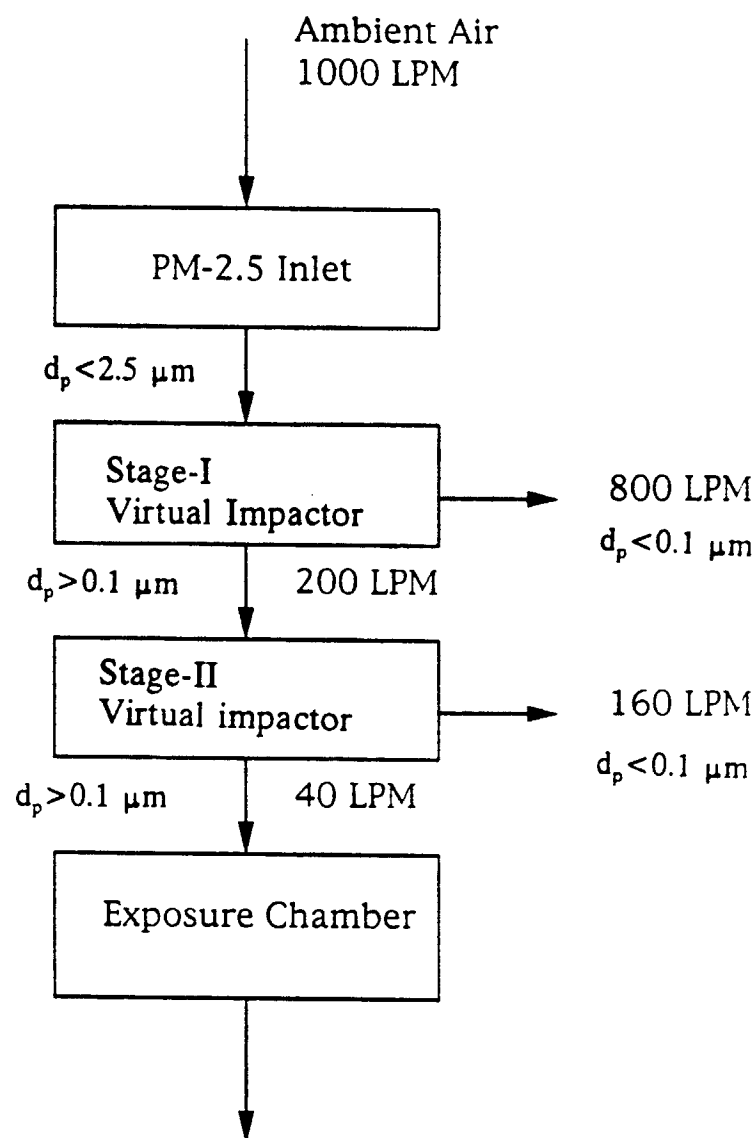


FIGURE 2. Block diagram of the ambient particle concentrator.

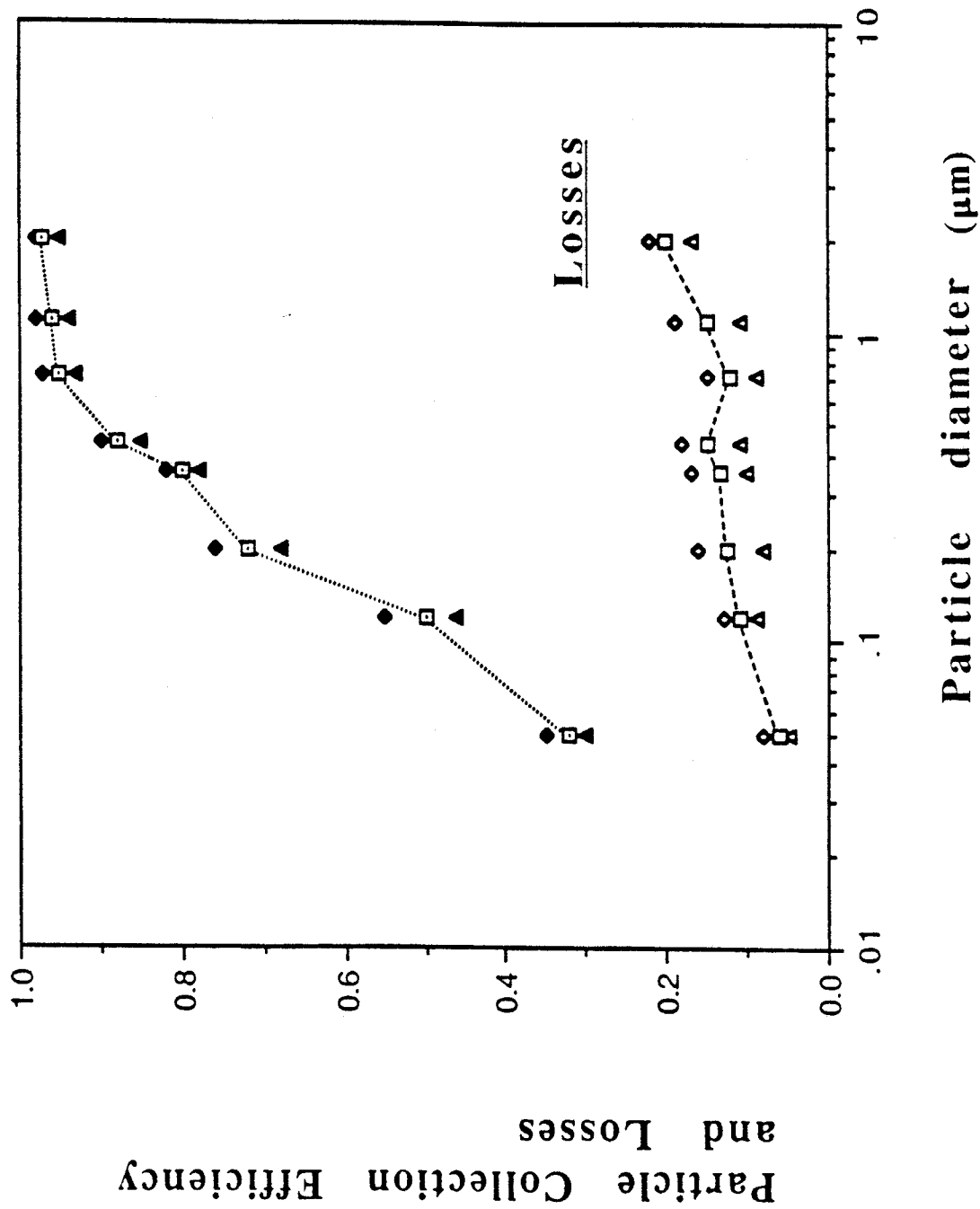


FIGURE 3. Collection efficiency and particle losses of stage II. The evaluation was done using fluorescent monodisperse aerosols in the size range 0.06-2.0 μm .

The Oronasal Airways: The Definer and Ignored Respiratory Zone of The PM-10 Regulatory Convention

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ABSTRACT

The particle penetration properties of the nasal and oral airways are the defining feature of the PM-10 convention. This definition implicitly assumes that only particles able to penetrate these airways can produce particle-associated health effects. Though not stated explicitly, this definition implies that no health effects resulting from exposure to particles are associated with these airways.

Some particles within the PM-10 envelope are deposited in the nasal and oral airways, as well as particles outside of the envelope (except for non-inhalable particles). Evidence for health effects of particles deposited in the oronasal (ON) airways is scant, primarily because most epidemiological and clinical studies have focused on the intrathoracic airways.

With improved techniques for detecting physiological changes and/or acute/chronic health effects in the ON airways, the role of these airways in providing data for regulation and control of particle exposure need further examination. Surveys of the incidence of nasal diseases indicate evidence for increasing occurrence of allergy, rhinitis and sinus infection in urban dwellers; these studies suggest that further investigation of the factors relating particle exposure to nasal disease are needed. There is evidence for variability of ON particle deposition efficiency whose relationship to health effects has not been studied.

INTRODUCTION

The definition of a PM-10 sampling convention is one of the key concepts of the current effort to regulate suspended particulate matter in the outdoor environment. A particle sampler which follows the PM-10 convention mimics the penetration properties of the human respiratory tract for particles able to pass through the oral passage and enter the intrathoracic airways. It follows in the historical evolution which included the concepts of dustfall, black smoke (in the UK) and total suspended particulate (TSP). It parallels developments in occupational health in which chemically unspecified particulate matter is measured according to one of the definitions of respirable particle matter [Lippmann(1989)].

A national ambient air quality standard (NAAQS) for suspended particulate matter, based on the PM-10 definition, has been adopted by EPA to protect the public from adverse health effects. This standard is derived from numerous health effect studies, referenced in the EPA Revision to NAAQS for PM(EPA, 1987), which includes animal toxicity studies, human clinical studies and epidemiological studies. Nearly all of the studies which are cited as critical focus on the tracheobronchial and alveolar zones(intrathoracic zones) of the respiratory tract. Recent human mortality studies have raised the issue of whether the NAAQS for PM-10 particulate matter is stringent enough [e.g., Dockery et al(1993)].

While this effort to examine the bases for the current particulate standard is being undertaken, it seems reasonable to consider some of the underlying assumptions of the PM-

10 sampling convention and how these might influence future health effect studies. The intent of this paper is to consider the regional respiratory dose of PM-10 particles and whether attention might also be directed to the extrathoracic airways (denoted herein as the oronasal zone, ON) as sites of adverse health effects.

The PM-10 Definition and ON Deposition

The PM-10 definition, or sampling convention, is an acceptance (or penetration) curve for the first stage of a sampling instrument whose acceptance percentages are given for specific aerodynamic particle diameters. It is, in essence, equal to the acceptance curve for thoracic particulate mass adopted by the American Conference of Governmental Industrial Hygienists (ACGIH) and the International Standards Organization (ISO) (shown in Table 1). It has a 50% acceptance at 10 μm diameter and a 0% acceptance cutoff at approximately 25 μm . This acceptance curve (as noted above) is intended to simulate the penetration of airborne particles through the human oral passage when breathing at resting to moderate rates. It is thus assumed that "mouth breathers" are a subpopulation which is at added risk of intrathoracic exposure to particulate matter because the oral passage is a less efficient inertial collector than the nasal passage.

From this definition, it follows that only particles which have access to the intrathoracic lung (during mouth breathing) are considered as being capable of producing adverse health effects. Particles which are totally excluded from these intrathoracic zones (aerodynamic diameters $> 25 \mu\text{m}$) are incapable of producing effects. Thus, if the criteria of health effects is access to the intrathoracic zones, it follows that these are the zones where attention should be focussed when looking for adverse health effects.

However, a typical deposition efficiency curve for the nasal passage (Fig. 1) shows that, for nasal breathers, some particles which are within the PM-10 "envelope", i.e., are counted as PM-10 mass, are deposited in the extrathoracic airways as well as in the intrathoracic zones. In the extreme, Fig. 1 shows that a typical nasal deposition curve has 100% collection at 10 μm aerodynamic diameter; thus, the 50% accepted particles of the PM-10 definition for this particle diameter are, on average (for nasal breathers), not accessible to the intrathoracic zones and cannot, therefore, produce effects in these zones. For smaller particle diameters, the nasal deposition curve ranges downward from 100%, and particles can be deposited, as above, in both the extra- and intrathoracic respiratory zones.

It can not be expected that a definition of an acceptance curve, such as the PM-10 definition, can simultaneously (1) perfectly account for the deposition characteristics of the respiratory zones, (2) allow for different breathing modes and (3) provide a sampler cutoff definition which can be readily simulated in an air sampling instrument. However, given the present definition, it cannot be assumed that only effects resulting from intrathoracic deposition need be considered as a basis for PM-10 standards.

Respiratory Dosimetry of PM-10 Particles

From the respiratory deposition curves proposed by the National Council on Radiation Protection and Measurements (NCRP) Task Group, Fig. 2 (Phalen et al, 1991), one may estimate the distribution of dose among the three respiratory zones for a given particle diameter within the PM-10 envelope. For example, for 3.0 μm , the percentage in the nasal

(ON), tracheobronchial (TB) and pulmonary (P) zones are, for resting breathing, respectively, 61%, 6% and 21%. While these values give the total mass of particulate in each zone, it is meaningful, as well, to "normalize" these values to the surface area of the deposition site for each zone. This normalization is based on the as-yet-unproven assumption that effects are related to surface concentration rather than just to total mass in a given zone.

Because of the mechanism of particle deposition (sedimentation) and the large surface area of the pulmonary zone, the surface concentration of particles in this zone is small. The total surface area of the TB zone is 4,731 cm² (Phalen et al, 1991). Even though not all of this is involved in deposition, mucociliary clearance tends to distribute this over a reasonable fraction of this area. The smallest area of deposition is in the nasal airway; Fig. 3 is a plot of relative local deposition in a replicate nose model (Swift and Bickert, 1994) which simulates an average nasal passage configuration. Approximately 50% of the deposition is confined to a region surrounding the nasal orifice whose surface area is estimated to be 5 cm² and which is not subjected to mucociliary clearance toward the pharynx. Thus, the local surface concentration of deposited particles in the anterior nasal region is relatively quite high compared to that in the TB and P zones.

Advances in Measuring Effects Related to ON Deposition

Most studies of exposure to PM-10 particles, either controlled human chamber exposure or epidemiological studies, have employed end points associated with intrathoracic effects. Measurements such as airway resistance, airway conductance, mucociliary clearance rate, forced expiratory volume (FEV), and forced vital capacity are well accepted tests of pulmonary function, as well as reproducible from one laboratory to another. Symptomatic changes are also generally related to the intrathoracic zones.

However, improvements in techniques for measuring changes in extrathoracic (especially nasal) function, as well as new methods for detecting changes which are indicative of health effects, suggest that greater attention should be given to this zone. These techniques include, by analogy to the lower zones, nasal resistance and nasal mucociliary clearance. A new technique which gives greater resolution to dimensional changes in the nasal airway than nasal resistance is acoustic rhinometry (Hilberg et al, 1989). This technique is attractive because of its simplicity, sensitivity, short time of measurement and essentially non-invasive character. It is possible to use this method with little children most of whom could not perform the maneuvers for nasal resistance measurements.

Another method for measuring acute effects of known nasal exposure to particles is the nose-in, mouth-out exposure technique which avoids intrathoracic exposure. This method has been employed for particle deposition studies and gives a reproducible and measurable particle dose to the nasal zone. It can be employed in conjunction with the above end points; its major disadvantage is its discontinuity and the difficulty of repeatedly performing the exposure to achieve a high level of exposure.

Conclusions

Particles within the PM-10 envelope are deposited both in the intra- and extrathoracic respiratory zones. Because of this, it cannot be assumed that the health effects associated with PM-10 particles are confined to the lower respiratory zones. The dosimetry of PM-10

particles, when viewed from a surface concentration perspective, is quite high in the nasal airway and may thus be considered as capable of producing measurable changes related to health effects. Surveys of the incidence of nasal diseases indicate increased occurrence of rhinitis, allergy and sinus infection which are thought to be exacerbated by particle exposure; such relationships merit further investigation (Mygind and Løwenstein, 1982). New techniques and improvements in existing techniques for measuring physiological and symptomatic changes in the nasal airway should lead to greater use in studies providing basic information for particle exposure standards.

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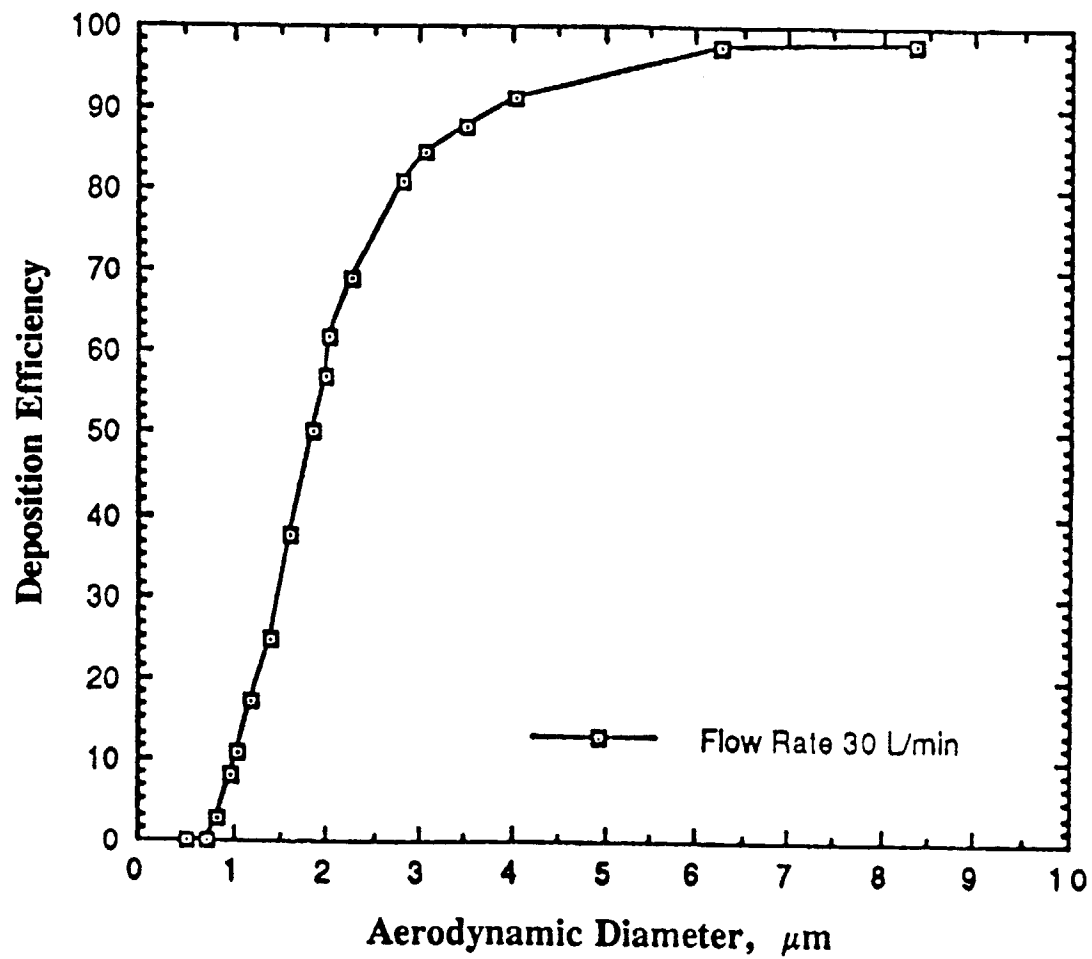


Figure 1. Typical Nasal Deposition Efficiency Relationship

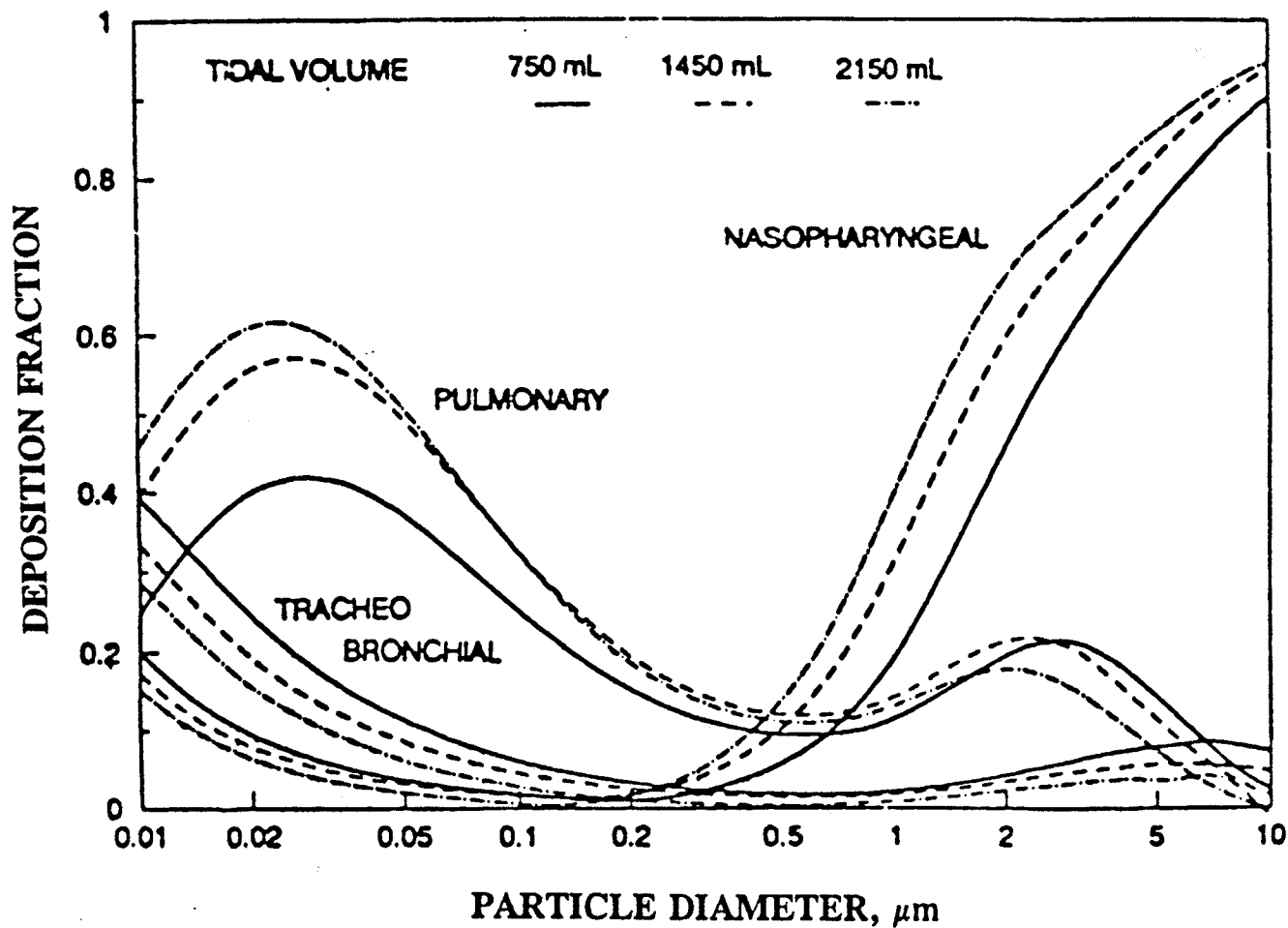


Figure 2. Respiratory Regional Deposition Fractions According to the Proposed NCRP Lung Model (Phalen *et al*, 1991).

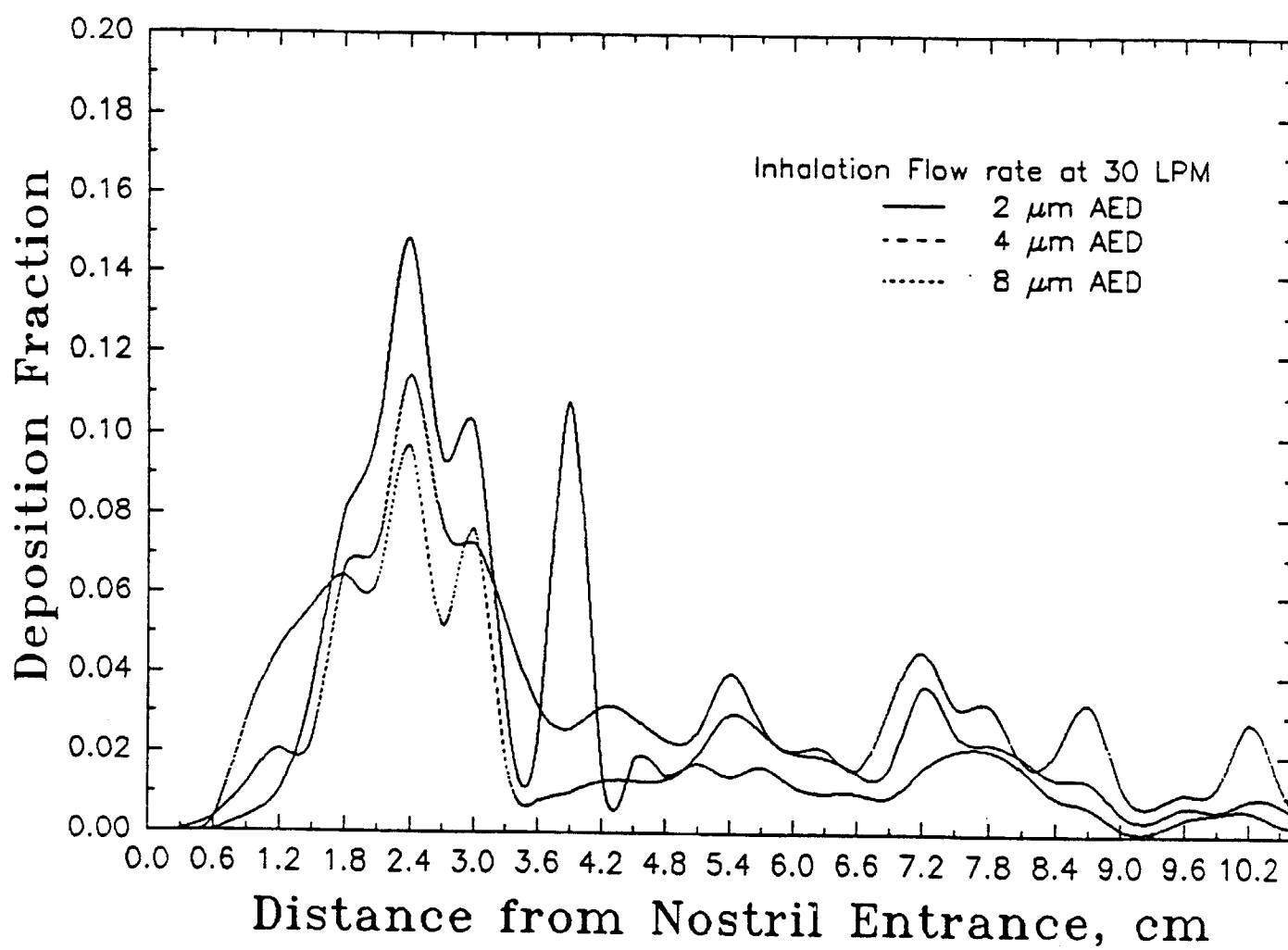


Figure 3. Local Nasal Deposition of Inertial Particles in an Adult Replicate Nasal Model.

**VARIATIONS IN PM10 CONCENTRATIONS
WITHIN TWO METROPOLITAN AREAS AND
THEIR IMPLICATIONS TO HEALTH EFFECTS ANALYSES**

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ABSTRACT

Temporal variations of PM₁₀ levels at multiple sites between 1985 and 1990 in two major metropolitan areas, Cook County, IL and Los Angeles County, CA, were characterized, and sensitivity of mortality/PM₁₀ associations to the choice of alternate sites was examined. In both cities, the correlation of PM₁₀ levels among multiple sites decreased as their distance increased. While averaging PM₁₀ concentrations over multiple sites generally improved the significance of PM₁₀-mortality associations, the highest PM₁₀/mortality association in Cook County was found for an individual site. In Cook County, the magnitude of the mortality association for the average of six PM₁₀ sites, as expressed as 'relative-risk' per 100 $\mu\text{g}/\text{m}^3$ PM₁₀, was similar (RR=1.06, 95% CI: 1.01-1.10) to those reported in other PM₁₀ studies with similar model specifications. However, the significance of regression coefficients for individual PM₁₀ sites varied considerably (t-ratios range: -0.62 - 3.30). Furthermore, every-6th-day subsamples of the daily data at a site in Cook County showed a wide range in the significance of regression coefficients (t-ratios range: -0.17 - 3.44). This variability of significance among the six sites may be partly due to their small sample sizes ($n \approx 300$), which raises concern regarding the potential for compromised statistical power of health effects analyses in 'short' study periods (< 6 years) at the current every-6th-day sampling frequency used for most PM₁₀ monitors in the U.S. Also, the qualitative site information available, such as land-use, location setting, and monitoring objective, did not show any coherent influence on the site's PM₁₀/mortality association's significance. Overall, it was found that the choice of PM₁₀ sites and sampling frequency can make a substantial difference in the calculated significance of such health effects time series analysis.

INTRODUCTION

Recent time-series epidemiological studies have reported associations over time between PM10 and mortality in various U.S. cities. Pope et al (1992) found significant increases in mortality associated with daily PM10 levels measured at a single site in Utah Valley during the period 1985-1989. Dockery et al (1992) found a significant association between PM10 measured at a single site in St. Louis and the total non-accidental mortality for the entire Metropolitan Statistical Area (MSA); they also reported a similar magnitude of association between PM10 levels monitored at a station in Harriman, Tennessee, and the mortality of surrounding counties during 1985-1986 study period. Schwartz reported (1993) an association between mortality in metropolitan Birmingham, Alabama and the average of PM10 measured at two sites (single site in 1985-1986; two sites in 1987-1988). In these studies, the PM10 concentrations analyzed were either from a single site, or the average of two stations.

Health effects outcomes such as mortality or morbidity are usually aggregated for a boundary (e.g., county, city, etc.) that has uneven population densities in relation to air monitoring stations. PM10 monitoring stations are not necessarily located to represent exposure within such a boundary. To further complicate the problem, levels of PM10, a chemically non-specific pollution index, can be more strongly influenced by local sources than regional secondary pollutants, such as sulfates. Thus, errors involved in the representativeness of population exposure covering a large area are inevitable if a single, or just a few, PM10 stations are used for health related exposure analyses. When measurement errors are random, they are assumed to bias the regression coefficient and correlation coefficient of health effects/pollution relationship downward (Snedecor and Cochran, 1980). However, the extent of such bias has not been quantified in past analyses, nor has it been clarified that measurement errors are in fact random, rather than systematic, probably due to the lack of data for multiple sites.

In this study, the variabilities of PM10 concentration at multiple sites in two large metropolitan areas, Los Angeles County, CA, and Cook County, IL, were characterized, and each

individual site, as well as the average of multiple sites, were examined for their associations with daily mortality of entire MSA areas.

DATA AND METHODS

Daily deaths for the Los Angeles County and Cook County were obtained from the National Center for Health Statistics for the period 1985-1990. Los Angeles County had the nation's largest population (8.3 million people) in 1986, and Cook County had the second largest (5.3 million people) (U.S. Bureau of the Census, 1988). Accidental deaths (International Classification of Diseases Codes ≥ 800) and deaths that occurred outside of each county of residence were excluded from this analysis.

Pollution data were obtained from U.S. Environmental Protection Agency's Aerometric Information Retrieval System (AIRS). The emphasis of this study is on the variabilities of PM₁₀ levels at multiple monitoring sites, but hourly carbon monoxide (CO) and ozone (O₃) observations were also obtained as reference pollution variables for health effects analysis. The averages of daily maximum 1-hr values of multiple CO sites (9 for Los Angeles and 3 for Cook County) and O₃ sites (8 for Los Angeles and 5 for Cook County) were then used for the analysis. In order to have a 'sufficient' sample size for the analysis, the sites that had more than four years, out of the six year study period, were used for the analysis. The application of this criterion resulted in four PM₁₀ sites for Los Angeles, and six PM₁₀ sites in Cook County. The hourly surface observations of meteorological parameters for Los Angeles Airport and Cook County's O'Hare Airport were obtained from National Climatic Data Center.

Each site was identified on the map, based on the address and latitude/longitude provided by the AIRS database, and the distance between each pair of sites was computed. While the database also provided qualitative site information such as adjacent land-use, location setting etc., this information was not used to exclude any site from this analysis, but rather used to aid in the interpretation of results. First, the site-to-site correlation was computed, and its dependency on the intervening distance was examined. Second, each site's time-series was regressed on: 1) seasonal

cycles (sine/cosine with periodicities of 2 yr, 1yr, 6mo, 1mo) ; 2) day-of-week dummy variables; and 3) a linear trend. This was done in order to examine the extent to which each component of the variation contributed to the difference/similarity among the time-series at the PM10 sites.

While the dependency on the above mentioned three factors may explain differences in the temporal fluctuations of PM10 levels observed within a metropolitan area, these factors are 'controlled' in most health effects analyses by either including the factors as dependent variables in the regression of health effect outcome on pollution, or by removing them (via regression) from dependent and independent variables prior to the regression or correlation analysis. Therefore, the sensitivity of using different PM10 sites in mortality analysis was examined via two approaches: 1) correlation analysis after removal of trends from both pollution and mortality; and 2) Poisson regressions of mortality on pollution and trends. In assessing the correlation between the pollutants and mortality, cross-correlation (correlation with lagged days) was also examined to identify the temporal lag structure of the association.

In addition to each individual PM10 site data, two possible types of averages of PM10 site data were computed: 1) average of all sites, as available; 2) average of all sites after each site's missing values had been filled in by regressing the site on the rest of sites' data, where available. The first average can be strongly influenced by a single site's levels when the other sites are missing, whereas the second average is, in effect, weighted to account for the average difference among sites even on days when only single site values are available.

Since a daily, rather than every-6th-day, sampling schedule was in effect at one of the Cook County sites, six every-6th-day subsamples of this series were analyzed, in order to examine any possible effect of every-6th-day subsamples vs. daily samples.

RESULTS

Figure 1a and 1b show the temporal fluctuations of two of the selected PM10 sites in Los Angeles and Cook County. The Azusa site has the strongest seasonal (long-wave) component, which peaks in the summertime. The Long Beach site's seasonal trend peaks in the wintertime.

The high short-term peaks tend to occur in the wintertime in all Los Angeles sites with the exception of Azusa, where they can also occur in late summer. In contrast to Los Angeles PM10, seasonal cycles are less obvious in Cook County PM10. The distribution of all the selected PM10 sites are compared in Figure 2a and 2b. The location setting and land use, as described in the AIRS database, are also indicated. PM10 levels are generally lower in Cook County than in Los Angeles.

Figure 3 shows the correlation coefficients (before detrending) of PM10 temporal fluctuations between each pair of PM10 sites, as a function of corresponding distance. The lowest association was that between Azusa and Long Beach sites, as expected from their opposite signs of seasonal trends. Detrending generally did not appreciably affect the correlations for both Los Angeles and Cook County sites, with the exception of Azusa-Long Beach case, in which the correlation increased from 0.36 to 0.57, making the correlations a better (negative) linear function of distance for Los Angeles. In both cities, correlation generally decreased as the distance between a pair of sites increased.

Table 1 shows the percent variance explained by season (sine/cosine), day-of-week, and linear trends for all the variables. In Los Angeles, seasonal cycles explained about half the CO and O₃ variance, while only 10-20 percent of the PM10 variance was attributed to seasonal cycles. In Cook County, an even lower percentage of variance of PM10 levels was explained by seasonal cycles, whereas as much as 58 percent of variance for O₃ was accounted for by seasonal cycles. A small, but often significant, percentage of PM10 variance was explained by day-of-week variables in both Los Angeles and Cook County. In most cases, PM10 was lowest on Sundays. While both Cook County and Los Angeles CO levels were lower on Sundays, O₃ levels in both cities were lower during weekdays. Only a small percentage of variance was ever attributed to the linear trend, and, when it was significant, the trend was downward over the study period.

In cross-correlation analysis (after detrending each series) of mortality vs. pollution variables, the strongest association between PM10 and mortality was observed on the same day in both Los Angeles and Cook County. In both cities, O₃ showed up to 2 day lagged associations with mortality. In Los Angeles, CO showed both associations and lag structure similar to those of

O₃, while in Cook County, CO was virtually non-significant. The temperature association was similar to that of O₃ in Los Angeles. In Cook County, the temperature association varied with season, being negatively associated with lagged mortality in the winter, but positively associated in the summer. Since PM₁₀/mortality associations were strongest on the same day in both cities, and the purpose of this study was to examine the sensitivity of the association to the choice of PM₁₀ sites, the comparison of strength of association was made for the same day only, as summarized in Table 1. In Cook County, the significance of PM₁₀/mortality associations ranged widely both among the six sites and among the six possible every-6th-day subsamples of site 2, but various average PM₁₀ series all showed significant associations with mortality. In Los Angeles data, one of the multi-site PM₁₀ showed the highest significance. The PM₁₀/mortality associations in Los Angeles were mostly weaker than those for Cook County, but 95% confidence intervals (via Fisher's Z-transformation) of these PM₁₀/mortality correlations overlapped.

Because there are more sites available in Cook County, the sensitivity of using alternate PM₁₀ sites, as well as alternate every-6th-day subsamples for one PM₁₀ site, in regression analyses was examined further in Cook County. In our basic model, mortality was regressed on the same day PM₁₀, temperature, and one-day lagged O₃, in addition to sine/cosine variables with 4 periodicities (2yr, 1yr, 6mo, and 1mo), day-of-week dummy variables, and a linear trend. More elaborate models, such as season specific models, etc., could be examined. For example, the application of seasonal specifications for two periods in which temperature effects are different in signs and lags (Oct.-Feb. lagged negative; Mar.-Sep. same day positive), both lower the significance and size of PM₁₀ coefficients somewhat (e.g., for average PM₁₀=100 μ g/m³, RR=1.05, 95% CI:0.97-1.13; and RR=1.03, 95% CI:0.98-1.09, respectively). However, the focus here is on sensitivity of results to the choice of PM₁₀ sites, and therefore only one basic model was used in this study. The correlations of the estimates of these covariates were generally small (PM₁₀ vs. ozone \approx -0.2; PM₁₀ vs. temperature \approx -0.2). Autocorrelation of the residuals for the daily PM₁₀ regression was of borderline significance (Durbin-Watson statistic=1.83 for n=1357). The autoregressive model resulted in non-significant autocorrelation of the residuals, but

slightly reduced the t-ratio of PM10 coefficient from 2.66 to 2.45. Sensitivity of results using various alternative models for Los Angeles data can be found elsewhere (Kinney et al., 1994), and a similar model-sensitivity analysis for Cook County is in progress by the authors. The regression results are summarized in Figure 4 in terms of the 'relative risk' calculated for increase in $100\mu\text{g}/\text{m}^3$ of PM10, an index often used in recent PM10/mortality studies (Dockery et al., 1992; Pope et al., 1992; Schwartz, 1993 and 1993b). It can be seen that the individual sites' significance, as well as those for the six possible every-6th-day subsamples of the site 2's PM10, range widely.

DISCUSSION

The results of this study indicate that the choice of PM10 sites can make a difference in the degree of significance reported in health effects analyses. Averaging over multiple PM10 sites appeared to help increase the level of the significance of mortality-PM10 associations, even when individual site(s) alone did not approach significance. However, in some cases, individual sites gave more significant results than for the multi-site average of concentrations. It is not clear, from this analysis, whether the sensitivity of the result is due to exposure errors in assigning different PM10 levels among the multiple sites to the entire metropolitan area population, or it is due to exposure errors added at an immediate vicinity of each site, possibly from local sources, or both. Because mortality data for smaller boundaries than county level were not available in this analysis, this issue could not be examined further.

The qualitative site description, such as land-use and location setting, available from the AIRS database (see Figure 2) appears to have no coherent influence on site's PM10/mortality associations. Therefore, without information on actual local source emission inventories, it is difficult to justify elimination of a site prior to health effects data analysis based on these qualitative site information. Relative distance of monitoring sites to population density in the study area may be also important, and systematic evaluation of this factor is needed.

Although the daily data at the site 2 and the average of 6 sites' PM10 levels in Cook County showed significant mortality associations, the range of significance of mortality associations for the six every-6th-day subsamples at site 2 was as wide as the range among the six sites. This result

raises a concern for a statistical power achieved by every-6th-day PM₁₀ sampling schedule for health effects analysis when a study period is 'short'. It should be noted that, in recent published studies on particulate matter/mortality associations (Schwartz and Dockery 1992; Schwartz and Dockery 1992b; Dockery, Schwartz, and Spengler 1992; Pope, Schwartz, and Ransom, 1992; Schwartz 1993), daily measurements of particulate matter, either TSP or PM₁₀, were available for at least one year period, and, when sampling schedule was every-6th-day, intervening missing values were predicted from humidity-corrected visual range (Schwartz, 1991). Thus, the current every-6th-day schedule used in most PM₁₀ monitors in the U.S. may compromise power required to detect any pollution/health effects relationships in many communities.

In both Los Angeles County and Cook County, the correlation of PM₁₀ levels among multiple sites decreased as their separation distance increased. Variability of levels at multiple sites need to be examined similarly for other pollutants. Depending on the physico-chemical characteristics and origins of a pollutant, sensitivity of the choice of sites in examining health effects is likely to be different from pollutant to pollutant. For example, sulfate levels tend to be regionally uniform, while acid aerosols and O₃ levels may vary even within a metropolitan area if other pollutants such as ammonia (to neutralize acid) or nitric oxide (to react with O₃) are not uniformly distributed in the region (Thurston et al., 1994). Thus, identification of a single causal pollutant, based simply on the strength of association with a health effect outcome without evaluation of attenuation/enhancement due to random/systematic errors in exposure estimates, may be misleading.

This study characterized the variability of PM₁₀ levels in two large cities, and assessed the sensitivity of observed associations to the choice of sites. However, we recognize that any reported associations of health effects cannot be attributable solely to particulate matter. An increasing number of epidemiological studies have shown associations between particulate pollution indices and health effect outcomes, but evidence from laboratory studies have been too circumstantial to conclude that respirable mass, without chemical specificity, causes health effects. Only after examining the various individual chemical constituents of particulate matter, the independent effects

and/or influence of other co-pollutants, and the downward/upward bias introduced by errors in measurement and spatial representativeness, can observational epidemiology do more than suggest causality for health effects of PM₁₀ exposures.

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Table 1. Summary of Percent Variance Explained by Trends and the Same Day Mortality/Pollution Associations

City	variable	Percent Variance Explained by Trends			The same day mortality association after detrending		
		Seasonal ^a	Day-of-week	Linear-trend	r	p-value	n
Los Angeles	mortality	47	0	0	-	-	2191
	temperature	26	0	1	0.121	0.0001	2191
	CO ₂ max	54	3	0	0.137	0.0001	2191
	O ₃ max	48	1	1	0.106	0.0001	2191
	PM10						
	site 1	20	4	0	0.073	0.169	356
	site 2	13	2	1	0.072	0.179	352
	site 3	10	2	1	0.049	0.377	330
	site 4	23	1	2	0.084	0.117	349
	average A ^b	11	2	0	0.077	0.120	405
Chicago	average B ^c	11	2	0	0.087	0.079	405
	mortality	22	1	0	-	-	2191
	temperature	78	0	0	0.061	0.0004	2191
	CO ₂ max	2	1	0	0.031	0.1466	2191
	O ₃ max	58	0	0	0.107	0.0001	2191
	PM10						
	site 1	6	2	1	0.199	0.0008	281
	site 2	7	1	2	0.121	0.0001	1251
	site 2_6 ^d _1 ^e	8	4	2	0.250	0.0001	246
	site 2_6 ^d _2 ^e	7	2	1	-0.006	0.9284	198
	site 2_6 ^d _3 ^e	11	5	1	0.073	0.2848	214
	site 2_6 ^d _4 ^e	8	5	1	0.106	0.1378	197
	site 2_6 ^d _5 ^e	10	3	2	0.115	0.1014	203
	site 2_6 ^d _6 ^e	14	4	2	0.106	0.1440	193
	site 3	8	4	0	0.024	0.6720	309
	site 4	7	4	6	0.145	0.0243	243
	site 5	9	6	0	0.110	0.0702	272
	site 6	11	4	0	0.159	0.0064	292
	average A ^b	7	1	1	0.102	0.0002	1357
	average B ^c	8	1	1	0.106	0.0001	1357
	average A ^b _6 ^d	10	4	0	0.154	0.0038	351
	average B ^c _6 ^d	9	4	0	0.147	0.0057	351

a: Sine/Cosine with periodicity of 2yr, 1yr, 6mo, and 1mo; b: Average of any available sites; c: Average of all the sites after each site's missing values had been filled in by regressing the site on the rest of sites' data where available; d: every-6th-day subsample; e: subsample number, 1=scheduled every 6th day, 2=the following day, etc.

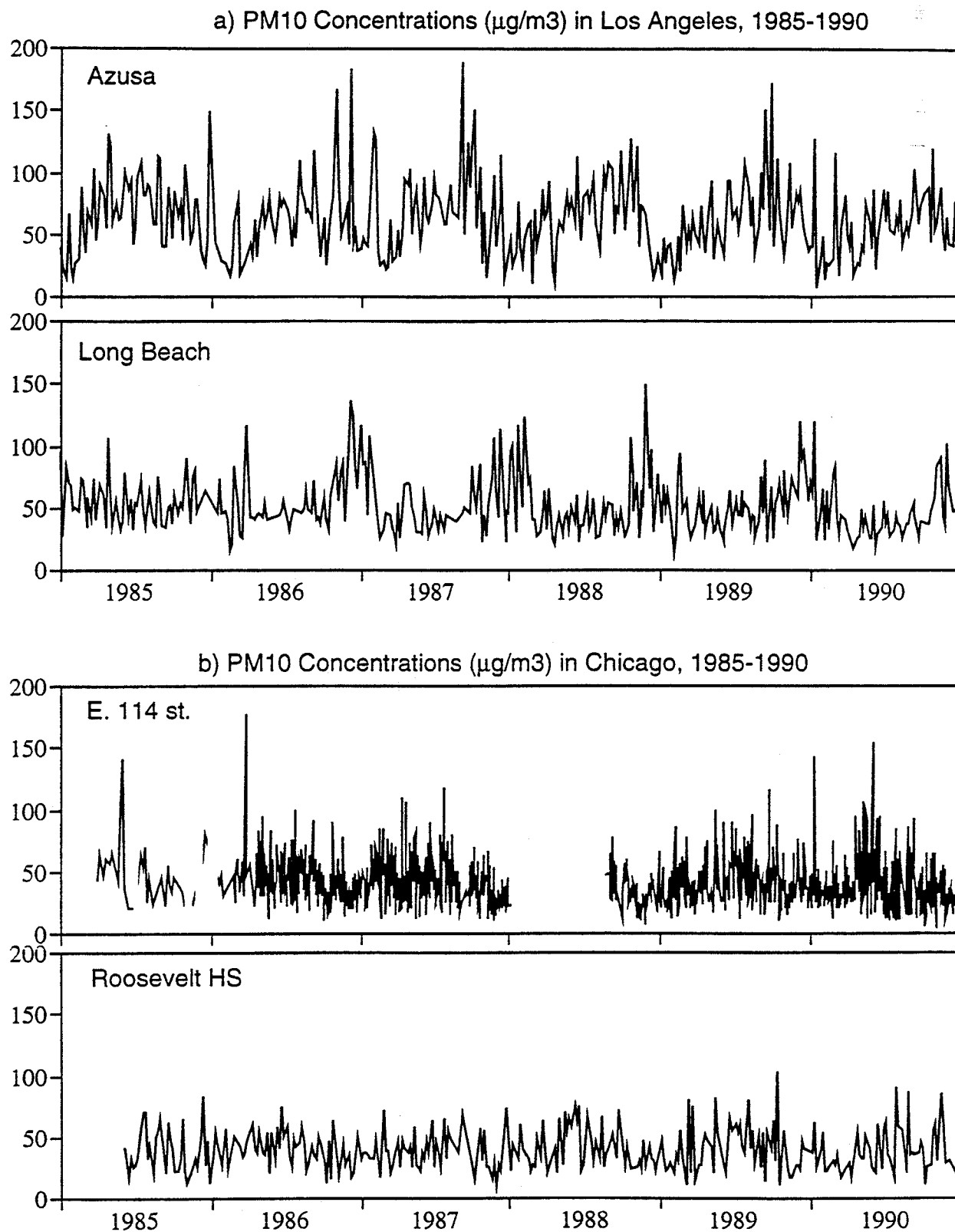


Figure 1. Time Series Plots of Los Angeles and Chicago PM10 levels at selected sites

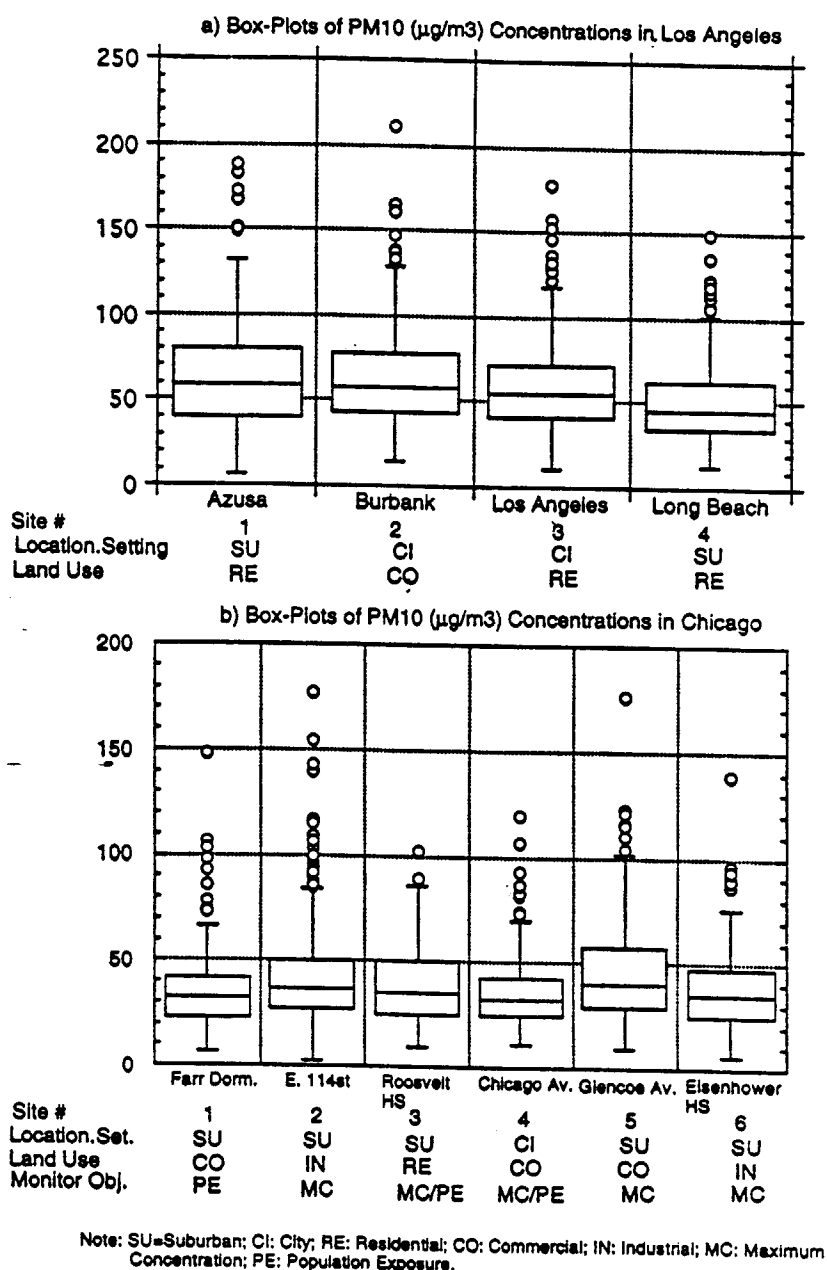


Figure 2. Box plots of PM10 levels for all the sites used in the study. (a): Box plots of PM10 concentrations ($\mu\text{g}/\text{m}^3$) in Los Angeles. (b): Box plots of PM10 concentrations ($\mu\text{g}/\text{m}^3$) in Cook County. The upper quartile, $Q(.75)$, and lower quartile, $Q(.25)$, of the data are the top and bottom of a rectangle, and the median is the horizontal line within the rectangle. The upper whisker is the largest observation less than or equal to $1.5(Q(.75)-Q(.25))$ plus $Q(.75)$. The lower whisker is the smallest observation greater than or equal to $1.5(Q(.75)-Q(.25))$ plus $Q(.25)$. Open circles are actual outside values.

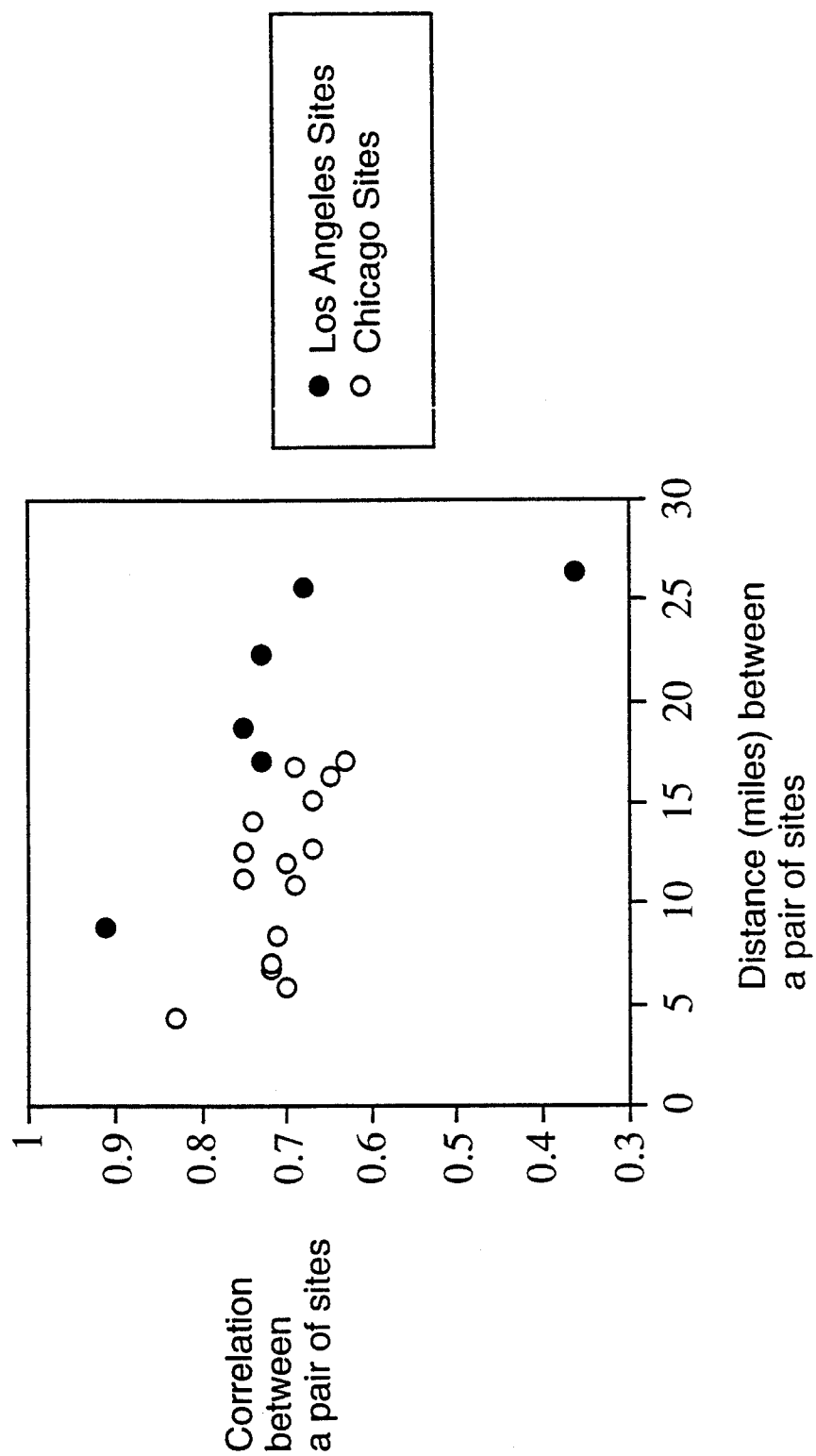
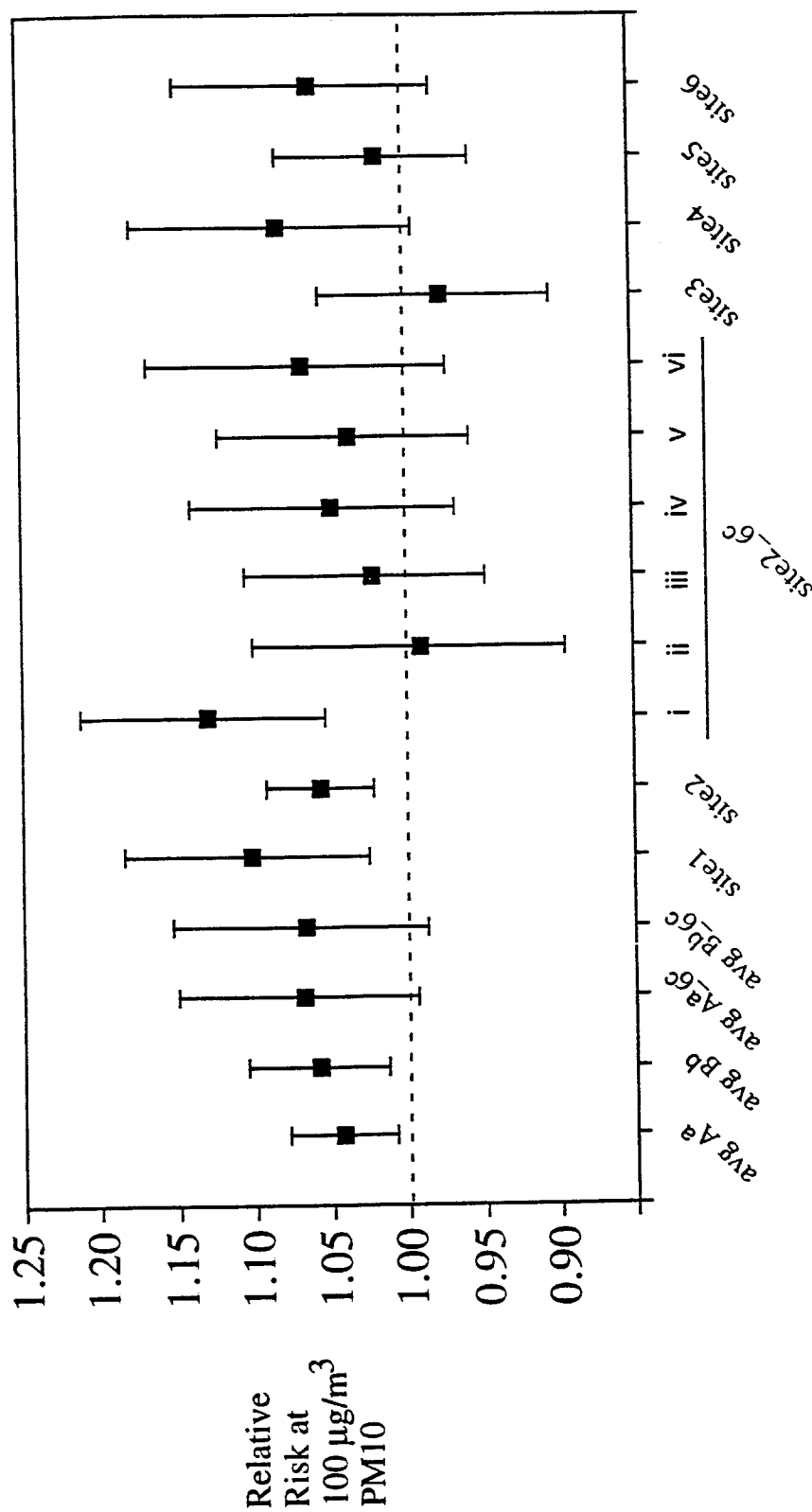


Figure 3. The Relationship between correlation of PM10 levels between sites vs. corresponding distance.



Note: a: Average of any available sites; b: Average of all the sites after each site's missing values had been filled in by regressing the site on the rest of sites' data where available; c: Every-6th-day subsample (i=the scheduled every-6th-day; ii= following day, etc.).

Figure 4. 'Relative Risk' and 95% confidence intervals for 100 µg/m³ PM10, calculated from Regressions of Mortality on PM10, O3, temperature, seasonal cycles, day of week, and trend. Chicago 1985-1990.